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The Mechanism of Iron (III) Oxidation of Glucose and Related Compounds

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THE MECHANISM OF IRON (III) OXIDATION OF GLUCOSE AND RELATED COMPOUNDS

A thesis submitted by

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SUMMARY

A study was undertaken to determine the mechanism by which ferric ion oxidizes sugars in the absence of oxygen. In 1M $HClO_4$ at 90°C., the reaction between ferric ion and glucose was zero-order with respect to the ferric ion concentration and somewhat less than first-order with respect to the glucose concentration. This and other evidence indicate that, at high acidities, reduction of ferric ion by glucose involves products from acid degradation of the sugar.

For the oxidation of glucose in 0.01M $HClO_4$ at 90°C., the dependence upon the ferric ion concentration was first-order after an induction period. The relationship between the reaction rate and the glucose concentration was best described by "Duke's kinetics," indicating oxidation via complex formation. Reaction kinetics and product analysis results suggested that glucose is oxidized first to the osone, with the latter undergoing a subsequent attack by ferric ion to give rise to two- and three-carbon carbonyl compounds. Several possible mechanisms are proposed for the occurrence of fragmentation products.

Oxidations of 3-O-methylglucose by ferric ion were similar to oxidations of glucose, except that, in the case of the methylated sugar, reactions did not exhibit induction periods and no fragmentation products were found. These results were taken as an indication that the C-3 methoxyl group prevents secondary oxidation of the primary product, the osone.

Oxidations of 2-deoxyglucose and 2,3,4,6-tetra-O-methylglucose were studied in order to verify the conclusion that the C-2 hydroxyl groups of glucose and 3-O-methylglucose are involved in reactions of the latter compounds with ferric ion. But, unexpectedly, both 2-deoxyglucose and 2,3,4,6-tetra-O-methylglucose turned out to be more reactive than glucose. Dehydration appears to be involved

in the reaction between 2-deoxyglucose and ferric ion. The reactivity of 2,3,4,6-tetra-O-methylglucose is unexplained.

A model compound study was carried out to assess the reactivities of the various functional groupings in glucose. Isobutyl alcohol, 2-butanol, trans-1,2-cyclohexanediol, cis-1,2-cyclohexanediol, 1,5-anhydroglucitol, and butyraldehyde were relatively unreactive toward ferric ion in comparison with glucose. Glycolaldehyde and glyceraldehyde reduced ferric ion very readily. The results of the model compound study support the hypothesis that it is the α-hydroxyaldehyde grouping in the acyclic form of sugars which is attacked by ferric ion.

Mechanistic studies were made of glycolaldehyde and glyceraldehyde oxidations by ferric ion. The oxidation of glycolaldehyde had a first-order dependence upon the ferric ion concentration in 1M $HClO_4$ (50°C.), but a zero-order dependence in 0.1M $HClO_4$ (70°C.) when the initial ferric ion concentration was 1×10^{-3}M. In 0.25M $HClO_4$, the reaction order with respect to the ferric ion concentration was found to be dependent upon the level of the initial ferric ion concentration, being zero-order at high, and first-order at low initial ferric ion concentrations. In both 1M and 0.1M $HClO_4$, oxidations followed first-order kinetics with respect to the glycolaldehyde concentration. The reaction product was shown to be glyoxal. The results are consistent with a mechanism involving enolization of the aldehyde to an enediol, followed by oxidation of the enediol by ferric ion.

Unlike glycolaldehyde, oxidations of glyceraldehyde showed a first-order dependence upon the substrate concentration at all acidities studied. The acid dependence was consistent with either a mechanism involving complex formation between glyceraldehyde and the ferric ion species, $Fe^{+3}_{aq.}$ or a mechanism involving direct attack by $FeOH^{+2}$.

Spectrophotometric evidence was obtained for the existence of complexes between ferric ion and glucose, glyceraldehyde, and trans-1,2-cyclohexanediol. The composition of the glucose-ferric ion complex was shown to be one mole of glucose per mole of ferric ion. But, for the other compounds, complexes involving two moles of substrate per mole of ferric ion seem to be formed at higher substrate concentrations.

Glucose and glycolaldehyde reaction mixtures were shown to initiate polymerization of methyl methacrylate, providing evidence for the formation of free radicals in oxidations of these compounds.

INTRODUCTION

EFFECT OF METAL IONS ON CELLULOSIC MATERIALS

Aging of paper and of cotton and linen textiles is a well-known, though little-understood phenomenon. Aging refers to loss of strength and/or development of color (reversion) which occur when cellulosic materials are exposed to the forces of nature. Aging can be brought about artificially through the use of elevated temperatures or ultraviolet irradiation.

Many factors have been postulated to be involved in aging. [See reviews of Rollinson (1) and Spinner (2)]. Recent work (1,3) suggests that the presence of certain metal ions may have a significant effect on the rate of reversion of cellulose in accelerated aging tests. Czepiel (3) studied the effects of aluminum, cupric, ferrous, ferric, cadmium, lanthanum, magnesium, and manganese ions on the thermal degradation of cotton linters. The first four ions were found to be increasingly good accelerators of degradation, but the latter four showed some tendency to inhibit degradation. Other investigators have also reported that metal ions affect cellulose degradation (4-10).

Czepiel suggested that accelerations of degradation by metal ions might be a result of proton production from hydrolysis of the hydrated metal ions. But he pointed out that, if this were true, the highly acidic aluminum ion should have accelerated degradation more than the less acidic ferrous and cupric ions.

This led Kraske to undertake a study of the effect of metal ions upon the rate of hydrolysis of glycosidic bonds (11). He showed that ferric sulfate accelerates the acid hydrolysis of cellobiose. Unexpectedly, it was found to also oxidize this glycoside, as evidenced by a reduction of ferric ion to the ferrous state. Several monosaccharides also reduced ferric ion. Kraske carried

out a brief study of glucose oxidation by ferric ion, but was unable to determine what the products of the reaction were, or to analyze the reaction kinetics.

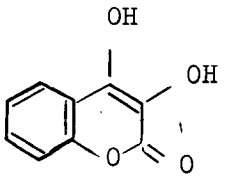
The present study was undertaken in order to elucidate the mechanism by which ferric ion oxidizes glucose. Then, with an understanding of what groups in glucose react with ferric ion, one ought to be able to predict where, in a cellulose molecule, ferric ion would be likely to attack. This might provide a basis for deciding whether acceleration of cellulose degradation by ferric ion involves oxidation. The work of Czepiel (3) and Kraske (11) indicates that hydrolysis is involved. But Spinner has observed (2) that colored substances in aged cellulosic materials seem to be formed from and contain carbonyl groups. This suggests that oxidation may contribute to the aging of cellulosic materials. Whether ferric ion's effect is due to an oxidative or a hydrolytic mechanism, or both, is the question that must ultimately be answered. The present study may not answer that question, but it is hoped that it will lead to further studies which will provide the answer.

OXIDATIONS BY FERRIC SALTS

OXIDATION OF STABLE ENEDIOLS BY FERRIC CHLORIDE

Arndt, Loewe, and Ayca (12,13) reported that compounds containing the α-keto enediol grouping, $-\underset{\underset{OH}{|}}{C} = \underset{\underset{OH}{|}}{C} - \underset{\underset{O}{\|}}{C} -$, complex with and reduce ferric ion. Complex formation is indicated by production of a blue to green color when ferric chloride is added to solutions of these compounds. Disappearance of the color accompanies reduction of ferric ion and oxidation of the enediol grouping to a dicarbonyl structure.

The nature of the complex was deduced from the behavior of the coumarindiol derivatives, I-III. Coumarindiol (I) gives a deep blue color with ferric chloride,



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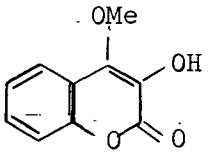

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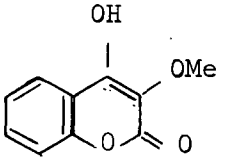
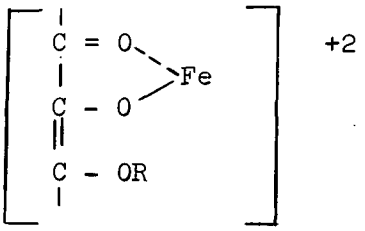


III

which fades with time due to reduction of the ferric ion; 3-hydroxy-4-methoxycoumarin (II) forms a color with ferric chloride, but the color does not fade; 4-methoxy-3-hydroxycoumarin (III) neither forms a color with nor reduces ferric ion. These observations suggested that the complex involves an enolic hydroxyl group which is in an α-position to a carbonyl group or a second hydroxyl group,



When R = H, the remaining enediol group can be oxidized, but no oxidation-reduction reaction occurs when R = CH_2. The authors stated that only the ferric ion which is not combined in the complex takes part in the oxidation of the enediol group.

OXIDATION OF ACETOIN BY FERRIC PERCHLORATE

Oxidation with ferric chloride is the standard method for the analysis of acetoin (14). Biacetyl is formed quantitatively and can be estimated as the nickel glyoxime complex.

Thomas, Trudel, and Bywater recently studied the mechanism of oxidation of acetoin by ferric perchlorate in aqueous perchloric acid solutions (15).

Under certain conditions the reaction was first-order with respect to both the ferric ion and acetoin concentrations. When methyl methacrylate was added to the reaction systems, it polymerized, showing the presence of free radicals. The following reaction mechanism was proposed:

$$Fe_{aq}^{+3} + CH_3COCH(OH)CH_3 \xrightarrow[\text{slow}]{k_1} Fe_{aq}^{+2} + CH_3C\dot{O}C(OH)CH_3 + H^+$$

$$Fe_{aq}^{+3} + CH_3C\dot{O}C(OH)CH_3 \xrightarrow{k_2} Fe_{aq}^{+2} + CH_3COCOCH_3 + H^+.$$

It was established that two moles of ferric ion are reduced per mole of biacetyl formed.

The rate of reaction was found to be directly proportional to the reciprocal of the acid concentration and to depend upon the ionic strength. This was explained as being due to the progressive hydrolysis of ferric ion which occurs at higher pH's, the various hydrated species of ferric ion being assumed to have different reactivities.

The authors noted that the kinetics of the reaction changed when the ratio of the ferric ion concentration to the acid concentration became too high. For example, oxidations of acetoin by 2×10^{-4}M ferric perchlorate gave pseudo-first-order plots (the acetoin concentration was greatly in excess of that of the ferric ion) in the acidity range from 1.0 to 0.03M $HClO_4$. But when the initial ferric ion concentration was increased to 2×10^{-3}M, the reaction ceased to obey pseudo-first-order kinetics at acid concentrations less than about 0.3M. First-order plots were curved over their whole lengths. The initial slopes were less than those observed with 2×10^{-4}M ferric perchlorate, but approached them as the ferric ion concentration dropped (as a result of the oxidation-reduction reaction). With 2×10^{-4}M ferric perchlorate, low initial rates were observed at acid concentrations below about 0.03M.

It was suggested that the departures from pseudo-first-order kinetics at relatively high ferric ion concentrations or low acid concentrations might be due to formation of the dimer, $Fe_2(OH)_2^{+4}$, under these conditions. But the authors pointed out that the dimerization constant would have to be several powers of ten larger than the accepted value to account for their results.

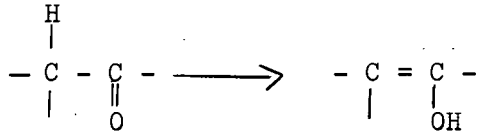
OXIDATIONS BY OTHER OXIDANTS

In the last twenty years, many studies have been carried out on the mechanisms of oxidation of organic compounds by transition metal ions and other oxidants. Some of the features which have been observed with other oxidants may be involved in ferric ion oxidations also.

Many oxidations of alcohols, glycols, aldehydes, ketones, and acyloins by metal ions have been shown to involve breakdowns of substrate-oxidant coordination complexes in the rate-controlling steps. Some of the metal ions for which oxidation *via* complex formation has been reported are Ce^{+4} (16), Mn^{+3}, V^{+5}, Co^{+3} (17) and Cu^{+2} (18). Oxidations of glucose by manganic sulfate (19) and ceric perchlorate (20) have been shown to involve disproportionation of a complex in the rate-controlling step. Levitt (21) has suggested that all organic oxidations by metal ions in acid media occur through complex formation.

The existence of free radical intermediates has been demonstrated for most oxidations of organic compounds by one-electron oxidants (such as ferric ion). This is not true in all cases, however. Oxidations of aldehydes, ketones, and nitroparaffins by alkaline ferricyanide do not appear to produce intermediate free radicals since addition of vinyl monomers to these systems does not result in polymer formation (22). The proposed reaction mechanism consists of complex formation between ferricyanide and anions of the substrates followed by attack on the complex by additional ferricyanide or by a similar complex.

In alkaline solution, oxidations of compounds containing a carbonyl function are often zero-order in oxidant and first-order in substrate and hydroxyl ion concentrations. This is usually interpreted as evidence that enolization,



is the rate-controlling step. The enol or enolate anion (formed by ionization of the enol hydroxyl) rather than the carbonylic form is the species which is attacked by the oxidant. Some of the reactions in alkaline media which are thought to involve enolic species are the oxidations of acyloins by mercuric halides (23); of glucose, acetoin (18), and benzoin (24) by cupric salts; of ketones by chromium (VI) oxide (25), of isobutyraldehyde by the free radical $\cdot ON(SO_3K)_2$ (26); and of aldehydes, ketones (22), and reducing sugars (27) by alkaline ferricyanide.

There is some controversy as to whether enolization is involved in oxidations in acidic media. Littler has concluded that oxidation of ketones by two-electron oxidants probably involves attack on the enol form (28), but that one-electron oxidants attack the keto form (29). However, the rate-determining step in the oxidations of aldehydes (30) and ketones (31) by manganic pyrophosphate and of isobutyraldehyde by V^{+5} (32) appear to be enolization since these reactions are zero-order in oxidant and first-order in substrate and acid concentrations.

ANALYSIS OF THE PROBLEM

Kraske (11), in his study of the ferric ion catalysis of cellobiose hydrolysis, discovered that cellobiose reduced ferric ion at 80-90°C. Glucose galactose, and ribose also reduced ferric ion, but ethylene glycol and inositol did not. The reactivities of the monosaccharides (ribose > galactose > glucose) were in the same relative order as amounts of aldehyde forms present in aqueous solution (33).

Kraske concluded that the glycol grouping is not involved in the reduction of ferric ion by sugars. Reactivity seemed to be related to the potential carbonyl group. By analogy to the α-keto enediols studied by Arndt, et al. (12,13), Kraske postulated that ferric ion oxidations of sugars occur through complex formation between ferric ion and their enediol forms.

Kraske's conclusion that glycols are not attacked by ferric ion is somewhat inconclusive. In addition to ethylene glycol and inositol, which gave no indication of reactivity toward ferric ion, Kraske conducted a reactivity test on sorbitol and found that reduction of ferric ion was about one half that for cellobiose. He attributed the apparent reactivity of sorbitol to the presence of a reducing compound, for which he obtained chromatographic evidence. But it was not shown that removal of the impurity eliminates reactivity of sorbitol toward ferric ion. A number of other metal ions including Ce^{+4} (16), V^{+5}, Co^{+3}, and Mn^{+3} (17) are known to oxidize glycols, and Kaden and Fallab (34) have stated that ferric ion attacks dihydroxy compounds according to the reaction:

$$Fe^{+3} + H_2Z \longrightarrow Fe^{+2} + H^+ + HZ\cdot.$$

Since ferric ion has a large positive charge, it is highly electrophilic. Therefore, it may be expected to attack a center of high electron density when

it oxidizes an organic molecule. In oxygenated compounds, such as sugars, oxygen atoms, by virtue of their unshared electron pairs, provide electronegative sites which may coordinately bond to ferric ion to form stable complexes or unstable transition state complexes. Thus, any oxygen atom in a sugar is a potential point of attack for ferric ion.

Cyclic (chelate) coordination complexes appear to be more stable than acyclic complexes (35,36). Since sugars have several pairs of oxygen atoms which are sterically capable of participating in chelate complexes, formation of this type of complex is quite likely in oxidations of sugars by ferric ion.

From a consideration of the various forms of glucose which may be in equilibrium in aqueous solutions, it seems that the following groups or groupings might be subject to oxidative attack by ferric ion:

1. monohydric secondary or primary alcohol groups
2. α-glycol groups
3. β-glycol groups
4. the aldehyde group
5. the gem-diol grouping of the hydrated aldehyde
6. the C-2 alcohol group
7. the ether linkage

MONOHYDRIC ALCOHOL GROUPS

Powerful metal ion oxidants such as Co^{+3}, Ce^{+4}, and manganic sulfate are able to oxidize monohydric alcohols, but the weaker oxidant, manganic pyrophosphate, is unable to do this (30). Since ferric ion is an even weaker oxidant than manganic pyrophosphate, it is unlikely that it would oxidize monohydric alcohols at an appreciable rate.

α-GLYCOL GROUPING

α-Glycols seem to be more easily oxidized than monohydric alcohols — perhaps due to formation of cyclic complexes. Manganic pyrophosphate, which does not oxidize monohydric alcohols, does oxidize α-glycols. However, Kraske's results with polyhydroxy compounds (11), though not conclusive, do indicate that ferric ion probably does not oxidize the α-glycol grouping.

β-GLYCOL GROUPING

There are a number of hydroxyl groups in glucose which are β to one another that appear to be close enough (from a study of Cenco-Petersen molecular models) to participate in a chelate complex. For most of these β-glycol pairings, close proximity is dependent upon glucose assuming unstable conformations with considerable steric hindrance. Because of this, these β-glycol groupings are probably less likely to complex with and reduce ferric ion than α-glycol groupings. However, the C-4 and C-6 hydroxyls can approach closer to one another than the α-glycol groups of glucose when glucose is in the stable C-1 conformation. Therefore, this β-glycol grouping might be more reactive toward ferric ion than α-glycol groups.

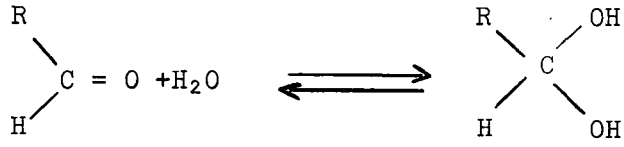
In the acyclic or open-chain form of glucose every hydroxyl group can approach any other hydroxyl group at least as closely as the distance between α-glycol groups. But sorbitol, whose structure is similar to that of the acyclic form of glucose, is relatively unreactive toward ferric ion (11) in comparison with glucose. So the oxidation of glucose probably does not involve reaction of the alcohol groups in the open-chain form.

ALDEHYDE GROUP

The aldehyde group of the acyclic form of glucose might be oxidized to a carboxyl group. Aldehydes are generally considered to be more susceptible to oxidative attack than are alcohols. But oxidations of aldehydes by one-electron oxidants, for example V^{+5} (37), $\cdot ON(SO_3K)_2$ (26), $Fe(CN)_6^{-3}$ (22), Mn^{+3} (30), and Cu^{+2} (38), generally do not give corresponding acids. The initial products seem to be the α-hydroxy aldehydes resulting from oxidation of the carbon adjacent to the aldehyde group (α-oxidation). In the case of ketones, α-oxidation has been reported for both one- (26,39,40,41) and two-electron (25,28) oxidants. These findings suggest that oxidation of the aldehyde form of glucose might result in oxidation at C-2 rather than oxidation of the aldehyde carbonyl to a carboxyl group.

GEM-DIOL GROUPING

In solution, aldehydes are in equilibrium with their hydrates, gem-diols (43):



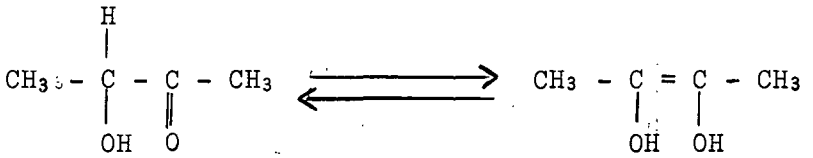
Oxidations of formaldehyde hydrate by V^{+5}, CO^{+3}, and manganic sulfate (44) appear to have the same mechanisms as oxidations of alcohols. Since it has already been pointed out that ferric ion is probably unreactive toward alcohols, it is unlikely that it would react with aldehyde hydrates either.

C-2 ALCOHOL GROUP

The hydroxyl at C-2 is unique in that it is adjacent to a carbonyl group in the acyclic form of glucose. From the reactivity of acetoin toward ferric ion (15), the α-hydroxycarbonyl grouping appears to be oxidized rather easily.

As mentioned earlier, the first products in oxidations of aldehydes and ketones are often α-hydroxycarbonyl compounds. These are usually difficult to isolate since, in most cases, they are oxidized more easily than the carbonyl compounds from which they were formed.

The ease with which acetoin is oxidized by ferric ion may be a result of chelate complex formation between ferric ion and the α-hydroxycarbonyl grouping or the 2,3-enediol grouping. The latter can arise by enolization of acetoin:



A 1,2-enediol can be formed from the aldehyde form of glucose by a similar process. This is believed to be the reactive species in oxidations of glucose by cupric salts (18) and alkaline ferricyanide (27).

THE ETHER LINKAGE

The ring oxygen in glucose has unshared electron pairs; thus it might engage in a coordination complex with ferric ion. Furthermore, it can approach fairly close to all the hydroxyls, raising the possibility of involvement in a chelate complex. But in the most stable C1 conformation, only the hydroxyls at C-1 and C-6 are close to the ring oxygen. Formation of a chelate complex involving C-1 and the ring oxygen is not as likely as one involving C-6 and the ring oxygen. A four-membered ring would be formed in the former case and a five-membered ring in the latter. The greater strain in the smaller ring would hinder its formation.

The possibility of reaction between ferric ion and the furanose form of glucose has not been considered in the foregoing discussions. There are two reasons for this. First, glucose is believed to exist predominantly in the

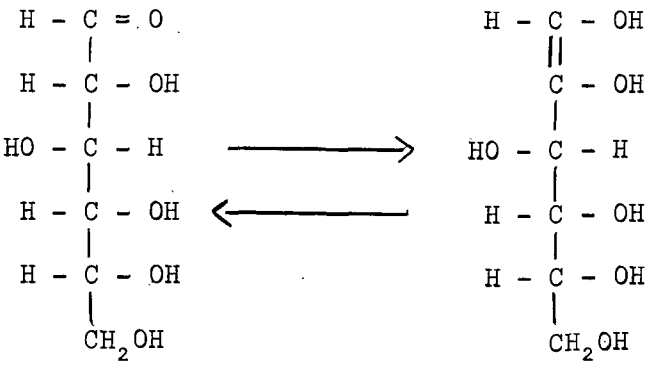
pyranose form in aqueous solution (45). And second, the main difference between the pyranose and furanose forms is that, with the latter, both the C-5 and C-6 hydroxyls can approach closely to the ring oxygen and the remaining hydroxyls (except that at C-2). But from the low reactivity of sorbitol toward ferric ion (11), it appears that close proximity of hydroxyls is not the factor which determines whether a compound will reduce ferric ion.

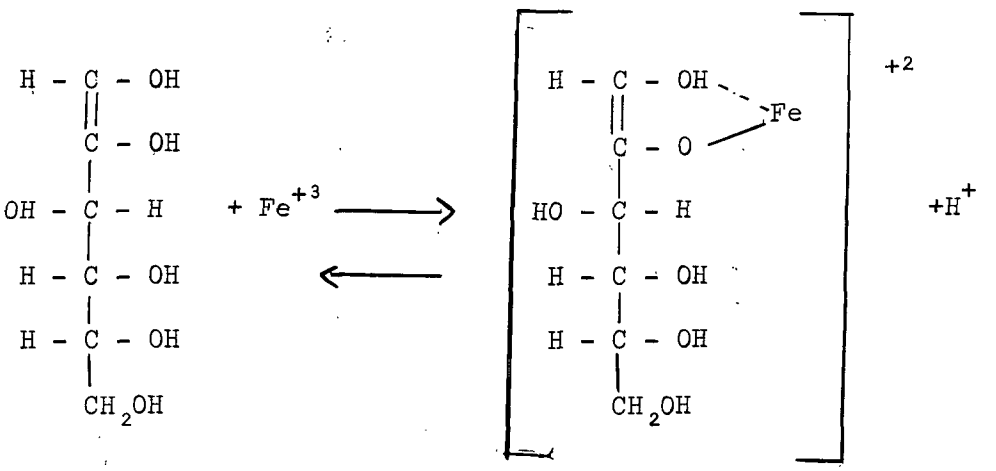
HYPOTHESES

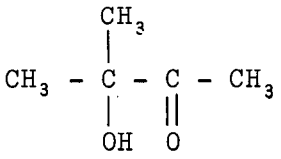
From a consideration of the potentially reactive sites in the glucose molecule, the most likely mechanism of oxidation by ferric ion appears to be oxidation of the C-2 alcohol group to a carbonyl group, probably *via* formation of a complex between ferric ion and the 1,2-enediol of glucose. This is the mechanism previously proposed by Kraske (11).

On the basis of free radical formation in the oxidation of acetoin by ferric ion (15), it is proposed that the ferric ion-glucose complex disproportionates to give ferrous ion and a free radical rather than being attacked by additional ferric ion to give stable products directly, as proposed for the oxidations of stable enediols by ferric ion (11,12).

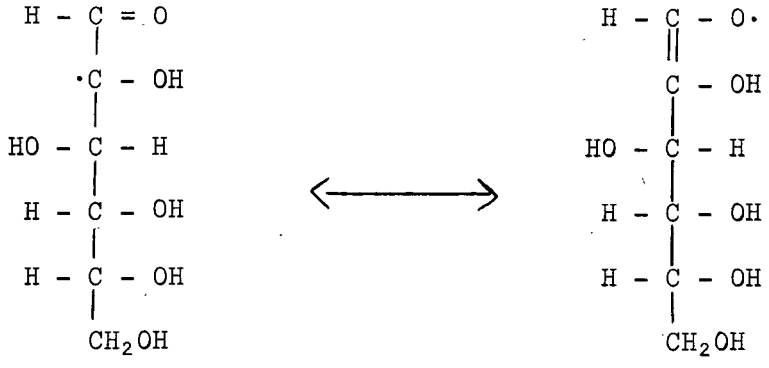
The proposed mechanism is as follows:







does not reduce ferric ion. Green has pointed out that this compound might be expected to be rather difficult to oxidize since a carbon-carbon bond must be broken in the oxidation (46). Since there is evidence (12,13) which indicates that enediols are especially reactive toward ferric ion, the preferred mechanism for glucose oxidation involves removal of a hydrogen from an enolic hydroxyl group rather than removal of the C-2 hydrogen. Actually, the two mechanisms are related in that the C-2 radical is in resonance with an enolic radical as shown below:



The initial product, D-arabino-hexosone (glucosone), would probably be oxidized rather easily by ferric ion since it can form a 2,3-enediol without having to open up into acyclic form. So, although the concentration of D-arabino-hexosone in the reaction mixture may be low in comparison to that of glucose, the concentration of its 2,3-enediol might be comparable to that of the 1,2-enediol of glucose (since the enediol of glucose is in equilibrium with the aldehyde form, which is only a small proportion of the total glucose concentration).

EXPERIMENTAL PROGRAM

The objective of the experimental program was to determine the mechanism by which glucose is oxidized by ferric ion. Accordingly, the focal point of the research program was a study of reaction kinetics, oxidation products, and complexing in aqueous solutions containing glucose, ferric perchlorate, and perchloric acid.

Because of the complex nature of the glucose molecule and the possibility of several forms coexisting in aqueous solution, it was anticipated that it might not be possible to determine some points about the reaction mechanism. For this reason a number of simpler model compounds were also studied. The purpose of the model compound study was twofold. First, to determine which of the potentially reactive groups in glucose are able to reduce ferric ion, and second, to determine the mechanism of oxidation of any groups which were found to be reactive. From the results of the model compound study, it was hoped to be able to propose a mechanism for the oxidation of glucose by inference.

Table I lists the potentially reactive groups in glucose and the model compounds which were used to test their reactivities. In addition to glucose and the compounds listed in Table I, 2-deoxyglucose, glyceraldehyde, 2,3,4,6-tetramethylglucose, and 3-O-methylglucose were studied for special purposes.

Experiments were conducted to determine whether oxidations of glucose and glycolaldehyde involve the formation of intermediate free radicals. Methyl methacrylate was added to reaction mixtures containing these compounds to determine whether polymerization would occur.

TABLE I

POTENTIALLY REACTIVE GROUPS IN GLUCOSE AND MODEL COMPOUNDS CHOSEN TO TEST THEIR REACTIVITIES

Group	Model Compound
Secondary alcohol group	Cyclohexanol and 2-butanol
Primary alcohol group	Isobutyl alcohol
trans-α-Glycol	trans-1,2-Cyclohexanediol
cis-α-Glycol	cis-1,2-Cyclohexanediol
β-Glycol	1,5-Anhydroglucitol
Aldehyde carbonyl	Butyraldehyde
Gem-diol	Butyraldehyde
α-Hydroxyaldehyde	Glycolaldehyde
Ether linkage	1,5-Anhydroglucitol

RESULTS AND DISCUSSION

COMPLEXING

SPECTRA OF COMPLEXES

While performing kinetic experiments it was noticed that a yellow-green color results when ferric perchlorate is added to solutions of polyhydroxylic compounds such as trans-1,2-cyclohexanediol, sorbitol, 1,5-anhydroglucitol, glucose, etc. This was interpreted as qualitative evidence for complex formation between these substrates and ferric ion. A spectrophotometric study was undertaken to obtain information on the nature of the complexes. Figures 1 and 2 show the spectra of ferric ion complexes of the substrates trans-1,2-cyclohexanediol, glucose, glyceraldehyde, and glycolaldehyde. Absorbance values were obtained by subtracting the sums of the absorbances of ferric ion and substrate solutions (unmixed) from those of the ferric ion substrate mixtures.

Figure 1 also shows similar data for a ferric ion-isopropyl alcohol mixture. The much greater absorbance of the ferric ion-trans-1,2-cyclohexanediol complex may be due to formation of a chelate complex with the α-glycol grouping. This would be expected to have a greater stability than an analogous complex involving a monodentate ligand (35,36).

COMPOSITIONS OF COMPLEXES

The method of limiting logarithms (47) was used by Kraske (11) to determine that one molecule of glucose is involved in the glucose-ferric ion complex. In the present study, an attempt was made to use the limiting logarithms method for determination of both the number of substrate molecules and the number of ferric ions involved in complexes of ferric ion with glucose, glycolaldehyde, glyceraldehyde, and trans-1,2-cyclohexanediol. But the method proved to be inapplicable under the conditions which were used. (See Appendix I.)

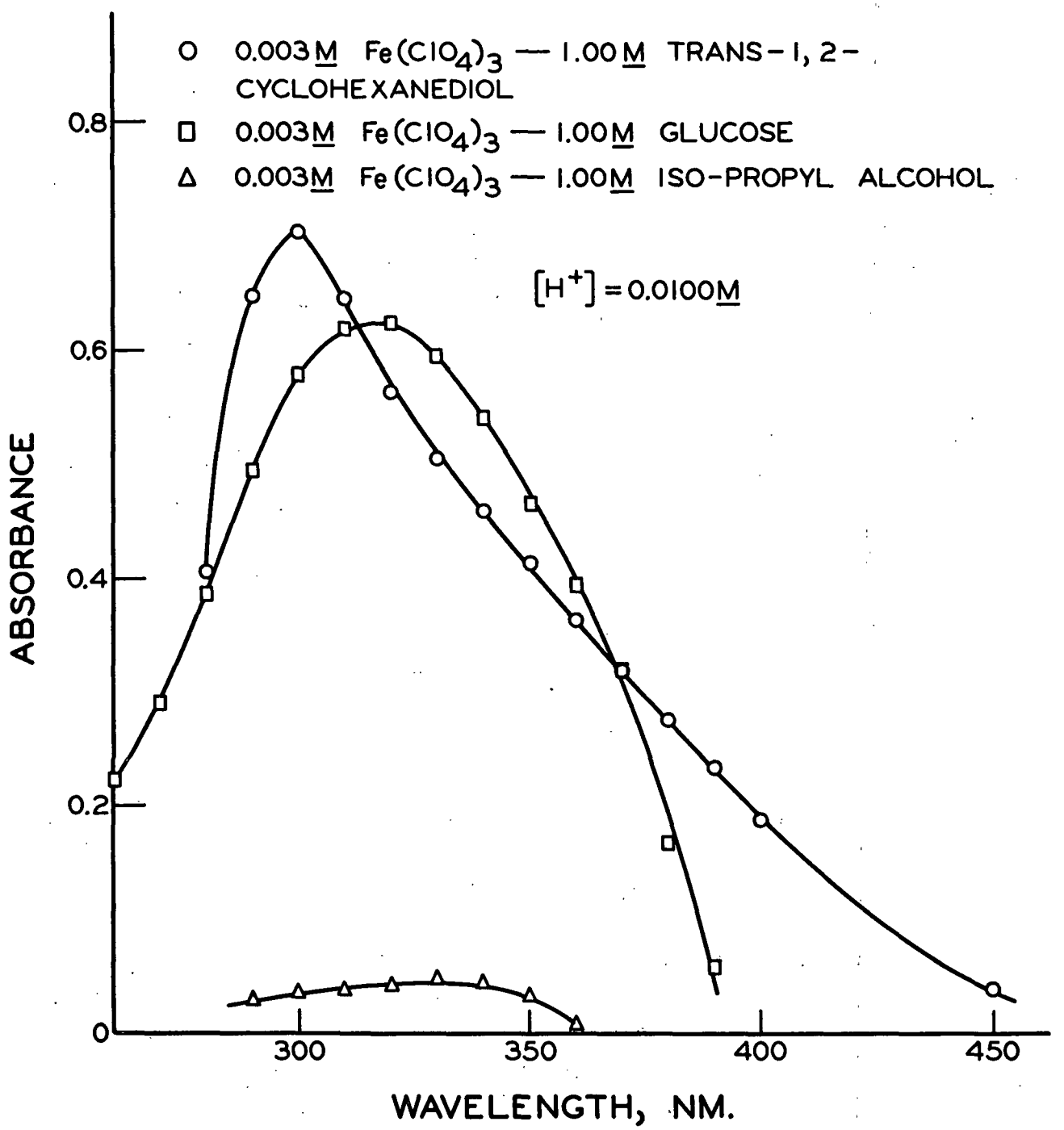


Figure 1. Absorption Spectra of the Ferric Ion Complexes of Glucose, trans-1,2-Cyclohexanediol, and Isopropyl Alcohol

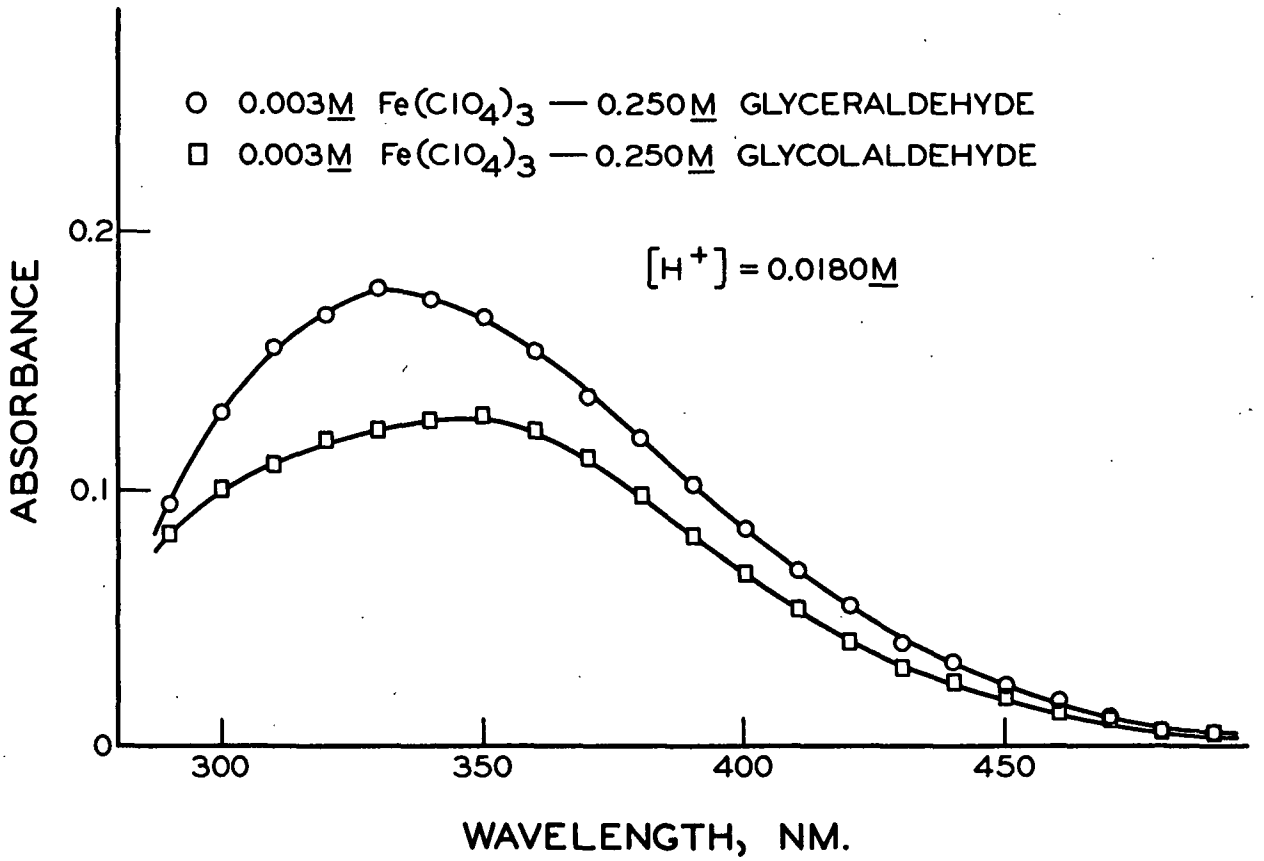


Figure 2. Absorption Spectra of the Ferric Ion Complexes of Glycolaldehyde and Glyceraldehyde

Ardon (48) used a spectrophotometric method for determining the composition of a ceric ion-ethanol complex. For the system,

$$M^+ + nL \underset{}{\overset{K}{\rightleftharpoons}} ML_n^+,$$

where

M^+ represents a metal ion of any charge,

L represents a ligand molecule,

ML_n^+ represents a complex ion,

n = the number of ligand molecules involved in the complex, and

K = the equilibrium constant for the complexing reaction,

when M^+ and ML_n^+ are the only absorbing species, the absorbance of the system is given by the equation,

$$A_T = (a_{ML_n^+})\,[M_T^+]\,X + (a_{M^+})\,[M_T^+]\,(1-X), \qquad (1)$$

in which

A_T = the total absorbance of the system,

$a_{ML_n^+}$ = the molar absorption coefficient of the complex,

X = the fraction of M^+ combined in the complex,

M_T^+ = the total metal ion (sum of the free and combined),

a_{M^+} = the molar absorption coefficient of the metal ion, and

[ ] represent concentrations of the enclosed species.

When L is in great excess compared with M^+, so that $[L] \approx [L_T]$ ($[L_T] = [L] + n[ML_n^+]$),

$$K = \frac{X}{(1 - X)\,[L_T]^n} \qquad (2)$$

Solving for $\underline{X}$ in Equation (2) and substituting into (1) gives, after inversion,

$$\frac{1}{A_T - [M_T^+]\, a_{M^+}} = \frac{1}{[M_T^+]} \left(\frac{1}{K\,(a_{ML_n^+} - a_{M^+})\,[L_T]^n} + \frac{1}{a_{ML_n^+} - a_{M^+}} \right) \qquad (3)$$

This equation can be used to determine the composition of a complex, since when $[\underline{M_T}^+]$ is kept constant and $[\underline{L_T}]$ is varied, a plot of $1/(\underline{A_T} - [\underline{M_T}^+]\underline{a_M})$ against $1/[\underline{L_T}]^{\underline{n}}$ will be a straight line if, and only if, the correct value of $\underline{n}$ is chosen and only one metal ion is involved in the complex. Alternatively, if $[\underline{L_T}]$ is held constant and $[\underline{M_T}^+]$ is varied, Equation (3) shows that a plot of $1/(\underline{A_T} - [\underline{M_T}^+]\underline{a_{M^+}})$ versus $1/[\underline{M_T^+}]$ will be a straight line as long as the complex involves only one metal ion (irrespective of the number of ligand molecules involved).

When a plot of $1/(\underline{A_T} - [\underline{M_T^+}]\,\underline{a_{M^+}})$ versus $1/[\underline{M_T^+}]$ is made for glucose-ferric ion mixtures (see Fig. 3) a straight line results, indicating that there is only one ferric ion in the complex with glucose. The same holds true for the substrates trans-1,2-cyclohexanediol, glyceraldehyde, and glycolaldehyde, as shown by Fig. 4 and 5.

For glucose, a plot of $1/(\underline{A_T} - [\underline{M_T^+}]\underline{a_{M^+}})$ against $1[\underline{L_T}]$ (Fig. 6) yields a straight line, showing that the complex is of the form, $\underline{ML}^+$. But analogous plots for trans-1,2-cyclohexanediol, glyceraldehyde, and glycolaldehyde (refer to Fig. 7) are curved. This indicates that ligand coefficients for these substrates unlike glucose, are not unity. Plots of $1/(\underline{A_T} - [\underline{M_T^+}]\underline{a_{M^+}})$ versus $1/[\underline{L_T}]^2$ are linear (see Fig. 8) except at low substrate concentrations. This suggests that complexes of the type $\underline{ML_2}^+$ are formed at higher substrate concentrations. This apparently is not the case with glucose, perhaps because of steric hindrance as a result of its greater size and degree of substitution.

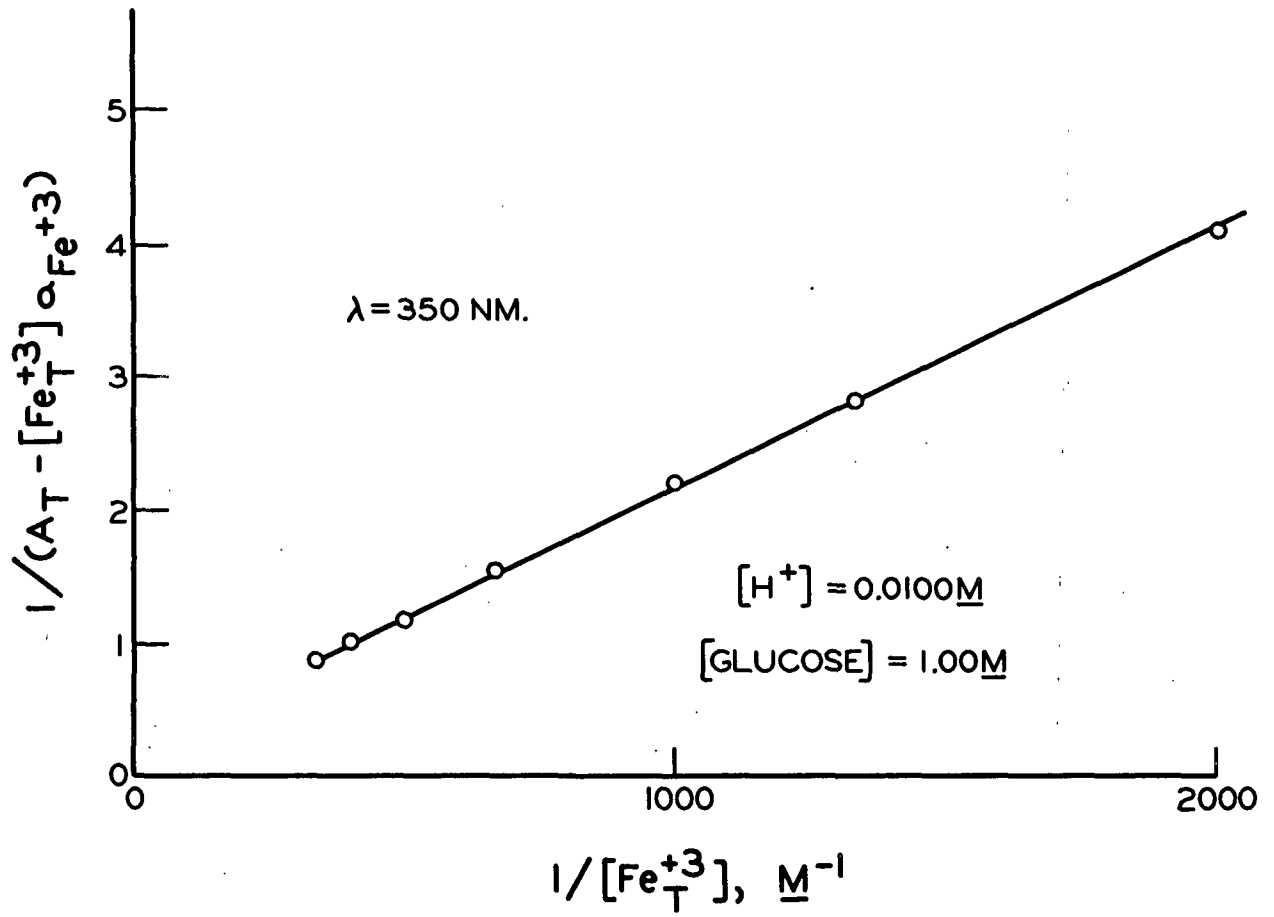


Figure 3. Test for a 1:n Ferric Ion-Glucose Complex

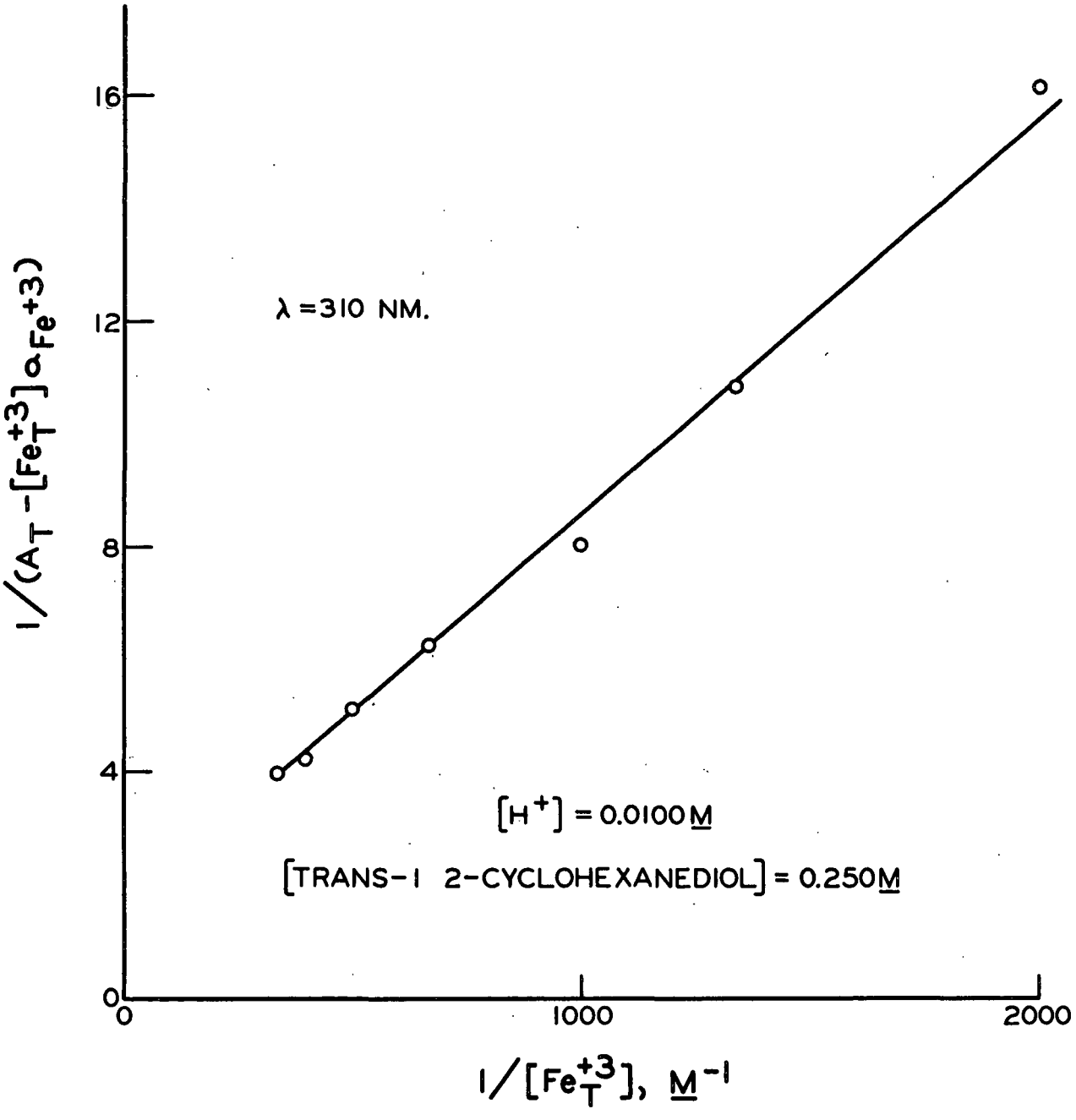


Figure 4. Test for a 1:n Ferric Ion-trans-1,2-Cyclohexanediol Complex

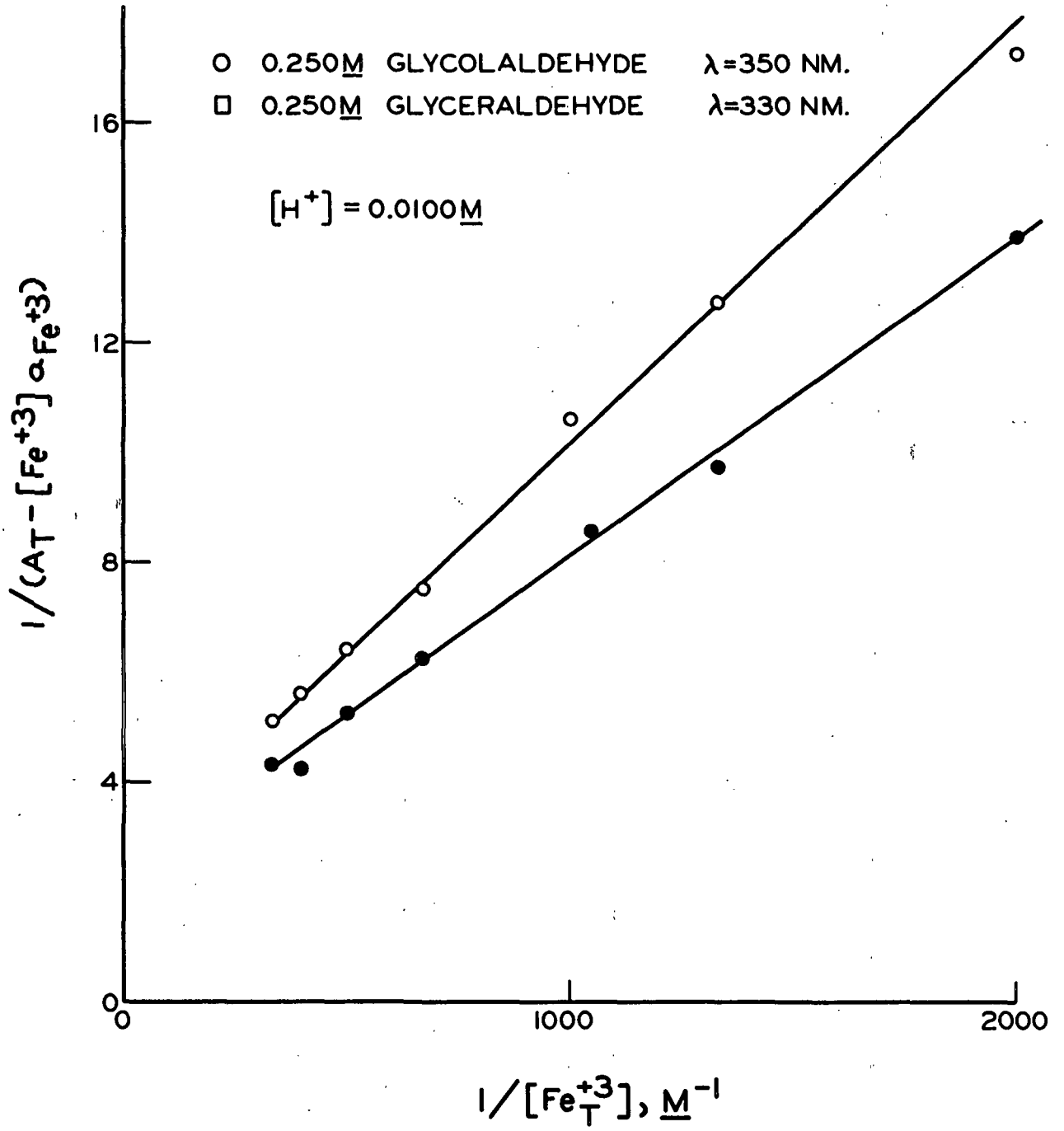


Figure 5. Test for 1:n Ferric Ion-Substrate Complexes for Glycolaldehyde and Glyceraldehyde

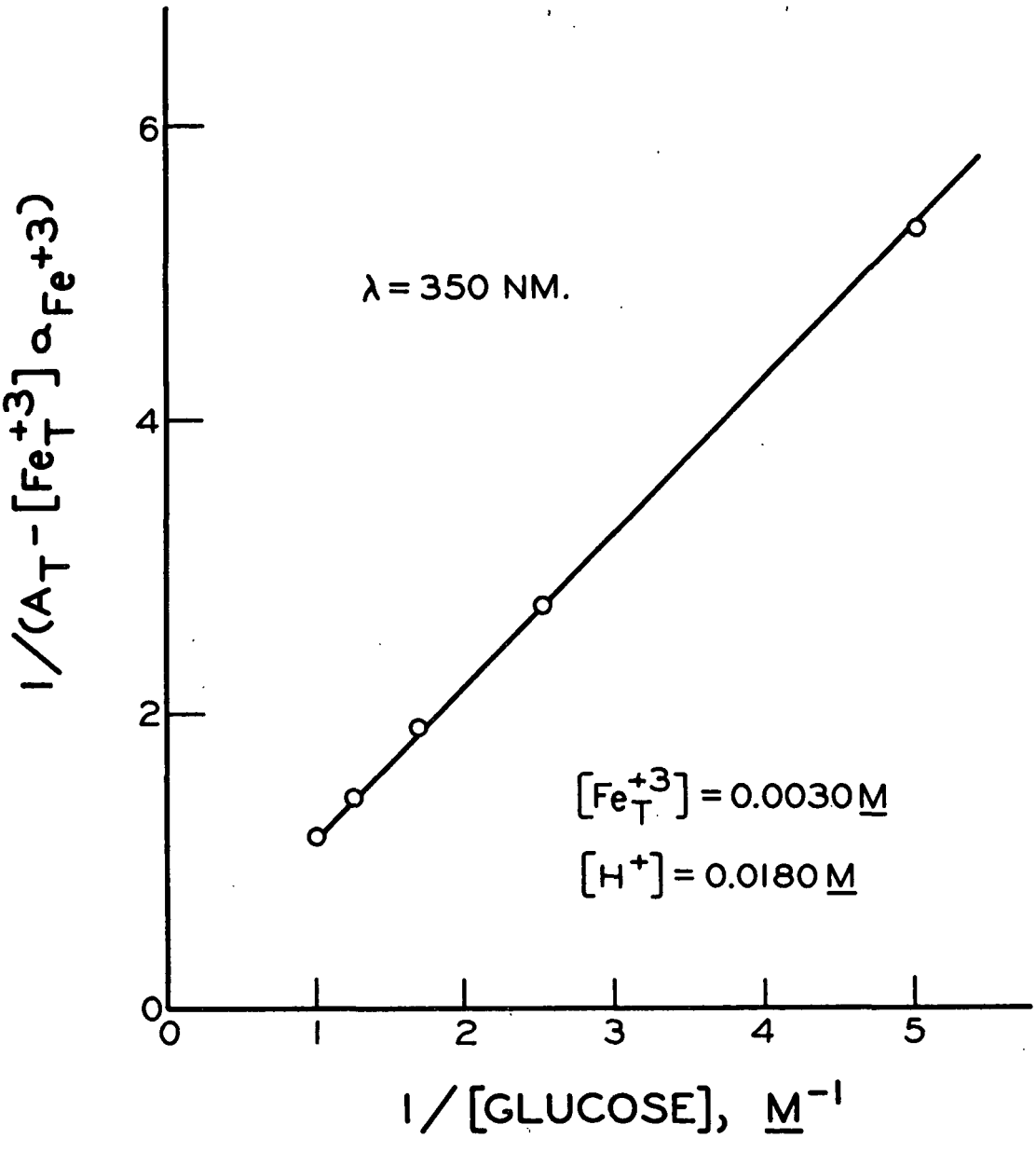


Figure 6. Test for a 1:1 Ferric Ion-Glucose Complex

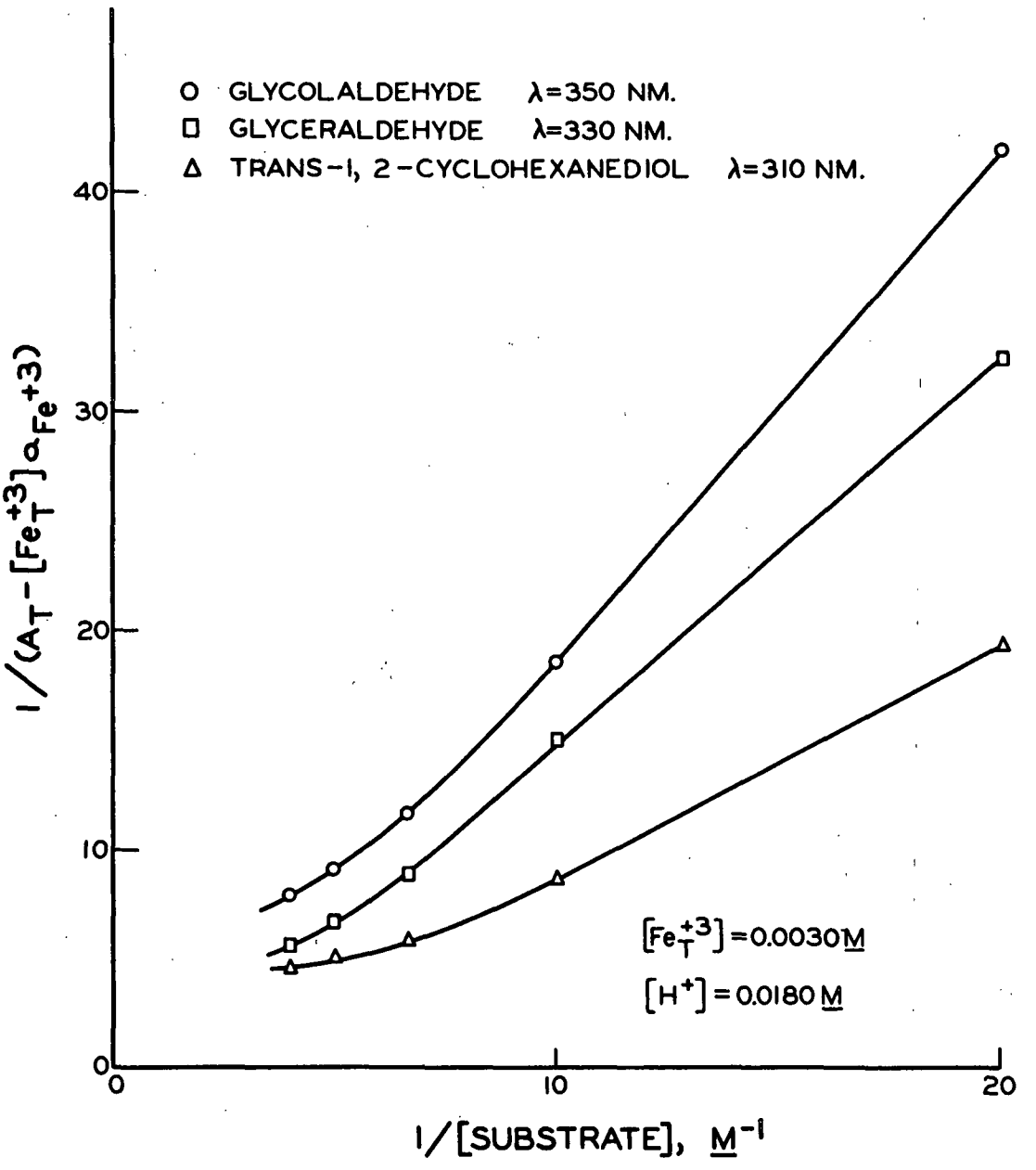


Figure 7. Test for 1:1 Ferric Ion-Substrate Complexes for Glycolaldehyde, Glyceraldehyde, and trans-1,2-Cyclohexanediol

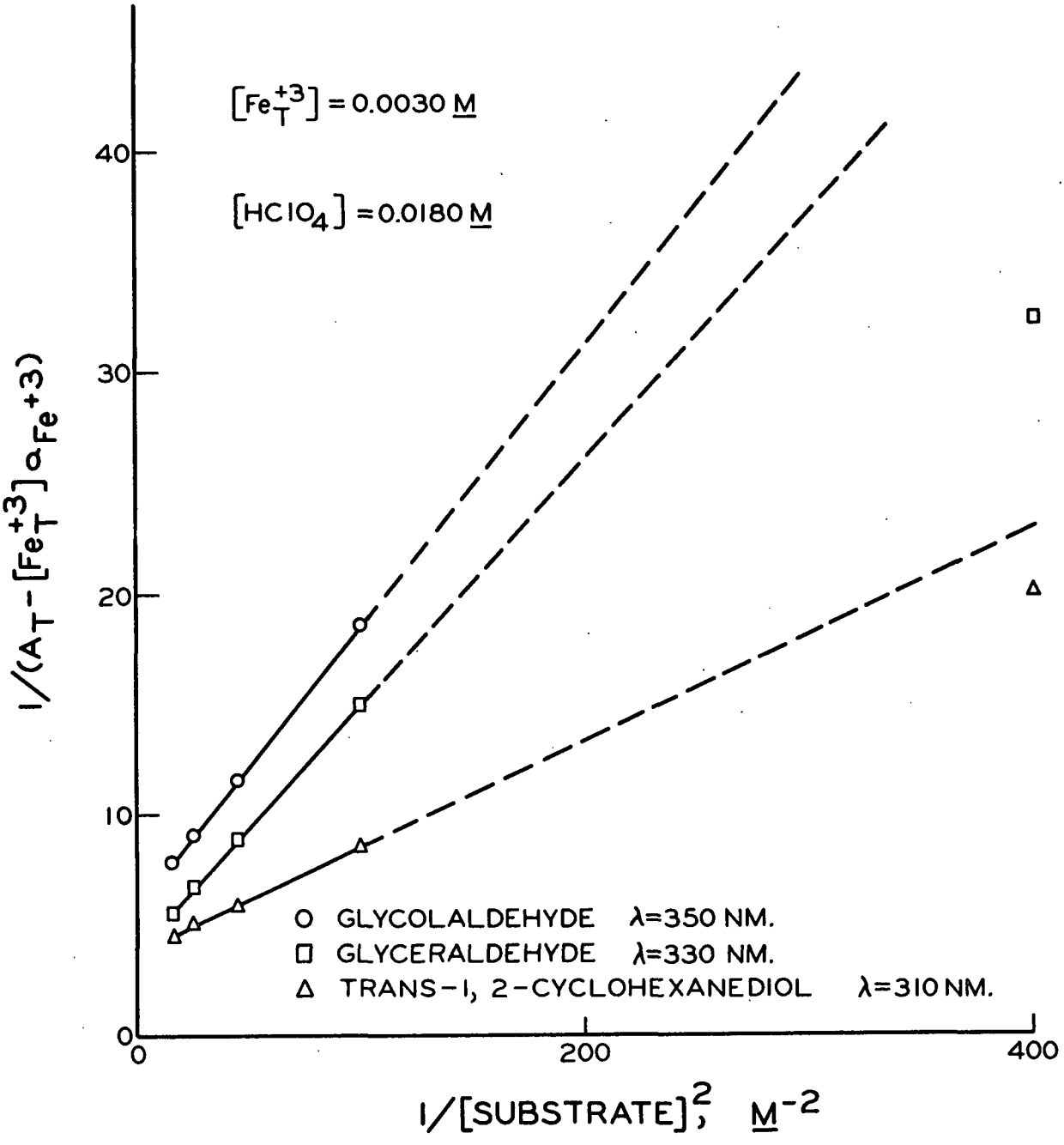


Figure 8. Test for 1:2 Ferric Ion-Substrate Complexes for Glycolaldehyde, Glyceraldehyde, and trans-1,2-Cyclohexanediol

POLYMERIZATION INITIATION

The existence of free radicals in a system may be demonstrated by placing a vinyl monomer in it. Vinyl polymerization is initiated by reaction of monomer with free radicals, so, if the latter are present, polymer should be formed. Thomas, Trudel, and Bywater (15) used this method to demonstrate that free radicals are formed in the oxidation of acetoin by ferric ion.

Methyl methacrylate (1%) was added to glycolaldehyde, glucose, and blank reaction mixtures, all of which were 0.001M in $Fe(ClO_4)_3$ and had ionic strengths of 1.0. A reaction mixture containing 0.01M glycolaldehyde and 0.01M perchloric acid initiated polymerization in 13-25 minutes at 70°C. In 1M perchloric acid, polymerization took place in less than five hours at room temperature, and almost immediately at 70°C.

For 0.1M glucose in 0.01M perchloric acid, polymerization occurred in about 40 minutes at 70°C. In 1M acid, polymerization had not taken place after 3 hours at 70°C., but when this solution was examined after 14 hours, there was polymer present.

Blanks containing no glycolaldehyde or glucose were also run. There was some polymer present in a blank 0.01M in perchloric acid after two hours at 70°C., but not nearly as much as in a similar solution containing glucose. A blank 1M in perchloric acid contained no polymer after 14 hours at 70°C. nor after four months at room temperature.

These results demonstrate that free radicals are formed in oxidations of glycolaldehyde and glucose by ferric ion. Polymerization initiation in the blank containing 0.01M perchloric acid may have been a result of direct attack of ferric ion upon methyl methacrylate.

OXIDATION OF GLUCOSE BY FERRIC PERCHLORATE

KINETICS

Effect of Acid Concentration

The effect of acid concentration upon the rate of oxidation of glucose (0.20M) by ferric perchlorate (0.001M) was studied at 88.6°C. by the automatic sampling and analysis method. (See pages 138-41 and Appendix II.) The ionic strength was maintained at 1.0M by addition of sodium perchlorate.

The results, shown in Fig. 9 and 10, reveal that reaction rate is decreased by an increase in acid concentration in the range from 0.01M $HClO_4$ to 0.25M $HClO_4$, but that above 0.25M $HClO_4$ this trend is reversed.

Thomas, Trudel, and Bywater (15) did not find this type of acid dependence in the oxidation of acetoin by ferric perchlorate. For acetoin, reaction rate decreased with an increase in acid concentration over the entire range of acidities studied (0.067M to 0.99M). Furthermore, reaction rate was linearly related to the reciprocal of the acid concentration. The authors attributed the acid dependence of the reaction to formation of a more reactive ferric ion species than Fe^{+3}_{aq}, probably $FeOH^{+2}$, by hydrolysis of Fe^{+3}_{aq}.

Glucose behaves similarly to acetoin in the range of acid concentrations from 0.01M to 0.25M in that the rate of oxidation decreases as the acid concentration is raised. The accelerating action of acid at higher concentrations must be the result of a reaction between the acid and glucose, if it is assumed that the reactive ferric ion species is the same in glucose and acetoin oxidations.

Reaction mixtures having acid concentrations higher than about 0.1M were yellow-colored after having been run, and the intensity of the yellow coloration

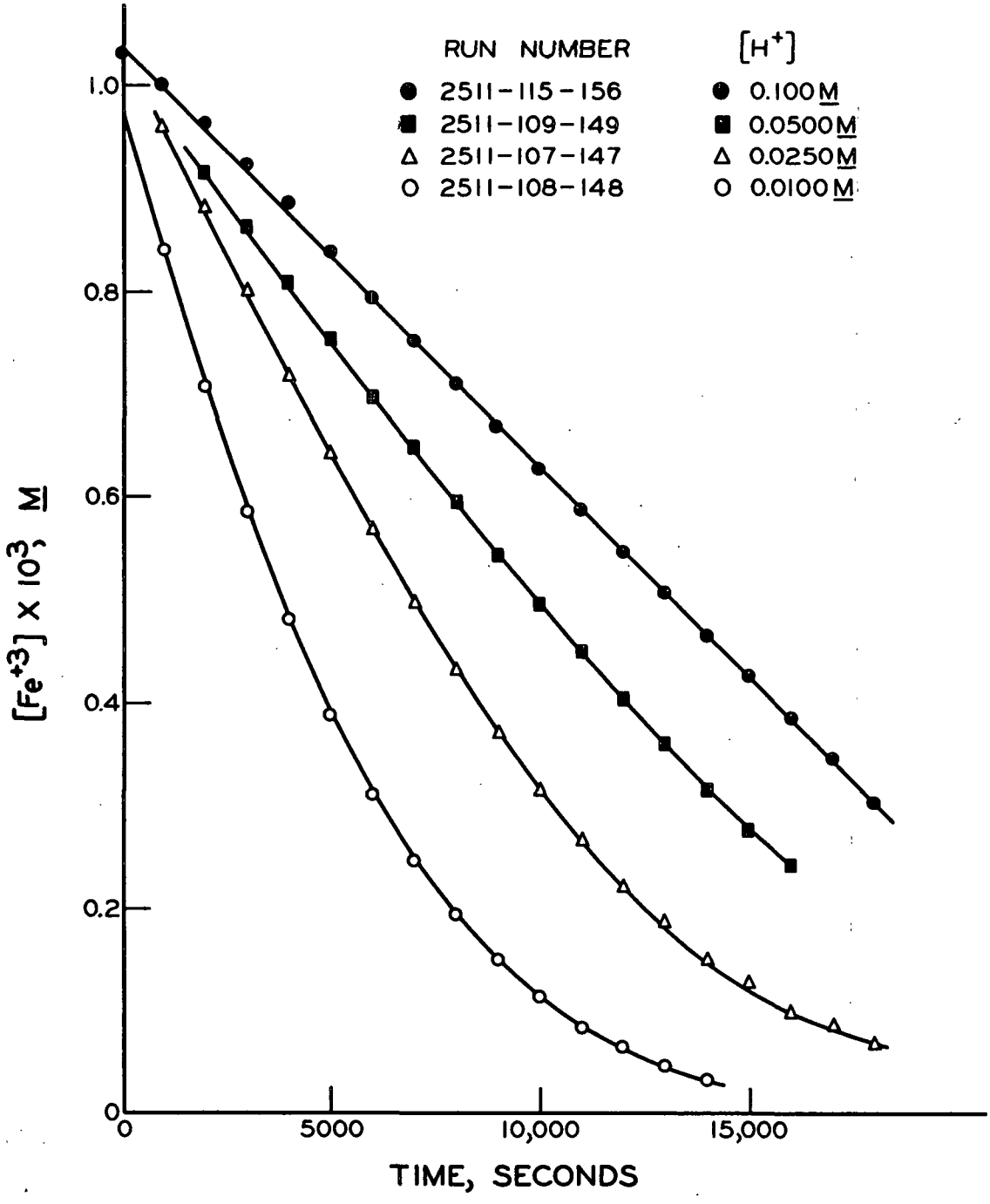


Figure 9. The Effect of Acid Concentration on the Oxidation of 0.200M Glucose by 10^{-3}M $Fe(ClO_4)_3$ at 88.6°C.

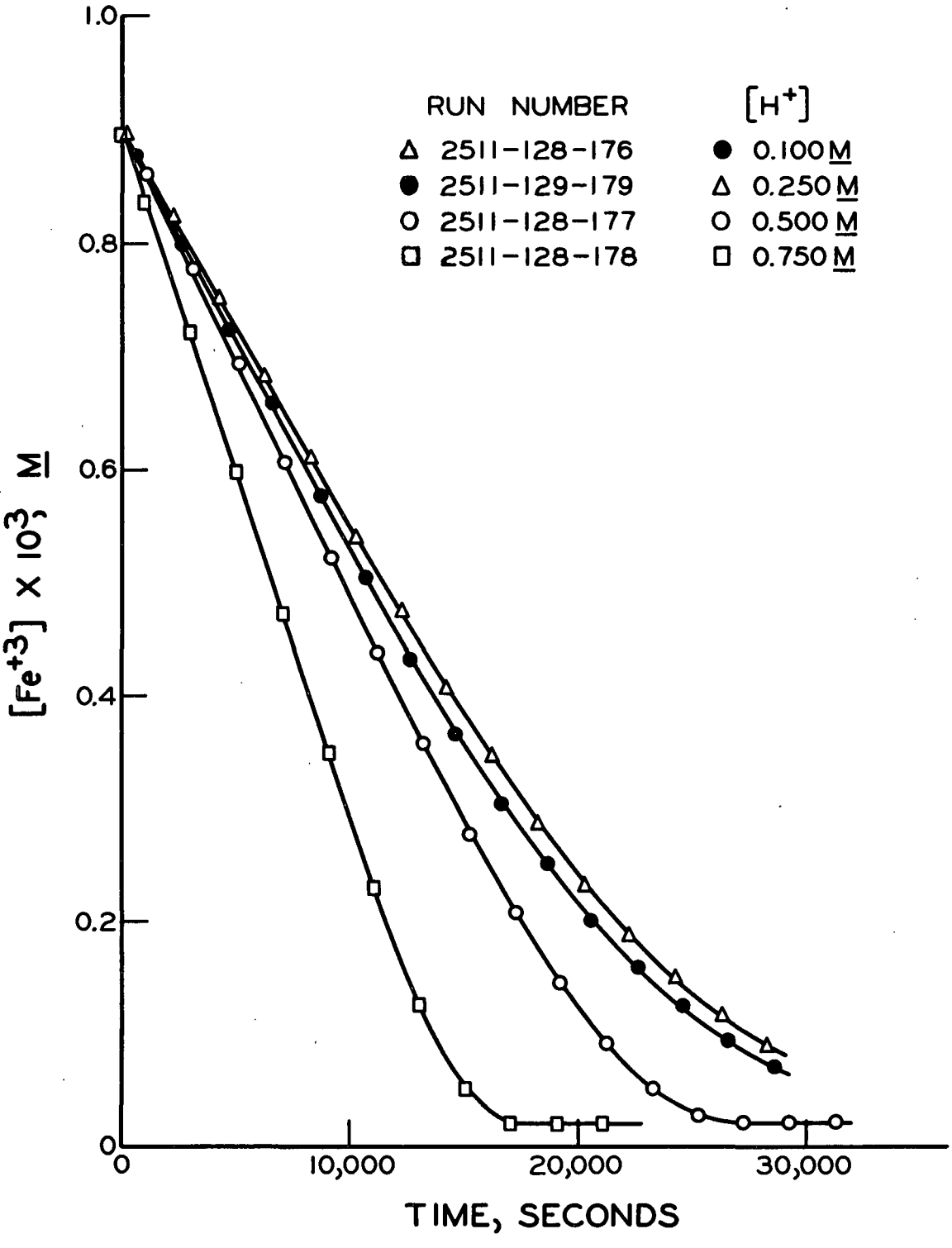


Figure 10. The Effect of Acid Concentration on the Oxidation of 0.200M Glucose by 10^{-3}M $Fe(ClO_4)_3$ at 88.6°C. — Time Axes Shifted so as to Put a Ferric Ion Concentration of 0.9 x 10^{-3}M at the Origin

was greater the higher the acid concentration. Dehydration is known to give rise to yellow-colored degradation products, probably by polymerization of 5-hydroxymethylfurfural (49). The following experiment was run to determine whether dehydration of glucose is significant under the conditions used in the oxidation studies.

Glucose (0.50M) in 1M $HClO_4$ was heated at 88.6°C. for 3.5 hours. Before heating, the solution was clear and colorless, but it was a deep brownish-yellow color after removal from the constant-temperature bath, indicating that glucose had undergone a significant amount of acid degradation. Addition of 25 ml. of saturated 2,4-dinitrophenylhydrazine in 2M HCl to an equal volume of the cooled glucose-$HClO_4$ solution gave 0.0120 gram of an orange precipitate. Thin-layer chromatography of this precipitate on silica gel with the solvent, benzene/tetrahydrofuran (4:1), showed the presence of at least nine components. The most abundant component had the same R_f as a 2,4-dinitrophenylhydrazone prepared from 5-hydroxymethylfurfural, confirming that dehydration of glucose can occur in glucose oxidation runs at higher acidities.

When a glucose solution which had been oxidized by ferric perchlorate in 1M $HClO_4$ was treated with 2,4-dinitrophenylhydrazine, a precipitate similar to that described above was obtained. But the amount of the component having the same R_f value as 5-hydroxymethylfurfural 2,4-dinitrophenylhydrazone was greatly reduced. This suggests that 5-hydroxymethylfurfural or a precursor to it is attacked by ferric ion. Reduction of ferric ion at higher acidities may be the result of a reaction between ferric ion and a dehydration product or intermediate rather than the result of a reaction between ferric ion and glucose.

Dependence upon Reactant Concentrations

Oxidations in 1M $HClO_4$

Kinetic experiments on all substrates, including glucose were carried out with the substrate concentration considerably in excess of the ferric ion concentration. Under this condition the substrate concentration is effectively constant during a run, changing only slightly as a result of reaction. Therefore, a rate equation such as

$$-d\,[Fe^{+3}]/dt = k\,[\text{Substrate}]^n[Fe^{+3}]^m, \qquad (4)$$

where the rate constant is k, and n and m are the orders of reaction with respect to substrate and ferric ion, respectively, may be replaced by the equation.

$$-d\,[Fe^{+3}]/dt = k'[Fe^{+3}]^m. \qquad (5)$$

In Equation (5), k' is a "pseudo" rate constant since the effect of substrate concentration is incorporated into it.

Equation (5) shows that, for any individual run, reaction rate is a function of the ferric ion concentration only. Therefore, m may be evaluated from the data of a single run. On the other hand, n may be determined by varying the substrate concentration in a series of runs and determining the effect of substrate concentration upon k' since

$$k' = k[\text{Substrate}]^n. \qquad (6)$$

Figure 11 shows an oxidation of glucose (0.10M) by ferric perchlorate (0.0010M) in 1.00M $HClO_4$ at 88.6°C. The rate of reaction is constant, independent of the ferric ion concentration, so the order of reaction with respect to ferric ion, m, is zero. (Note: The manual sampling and analysis method was used

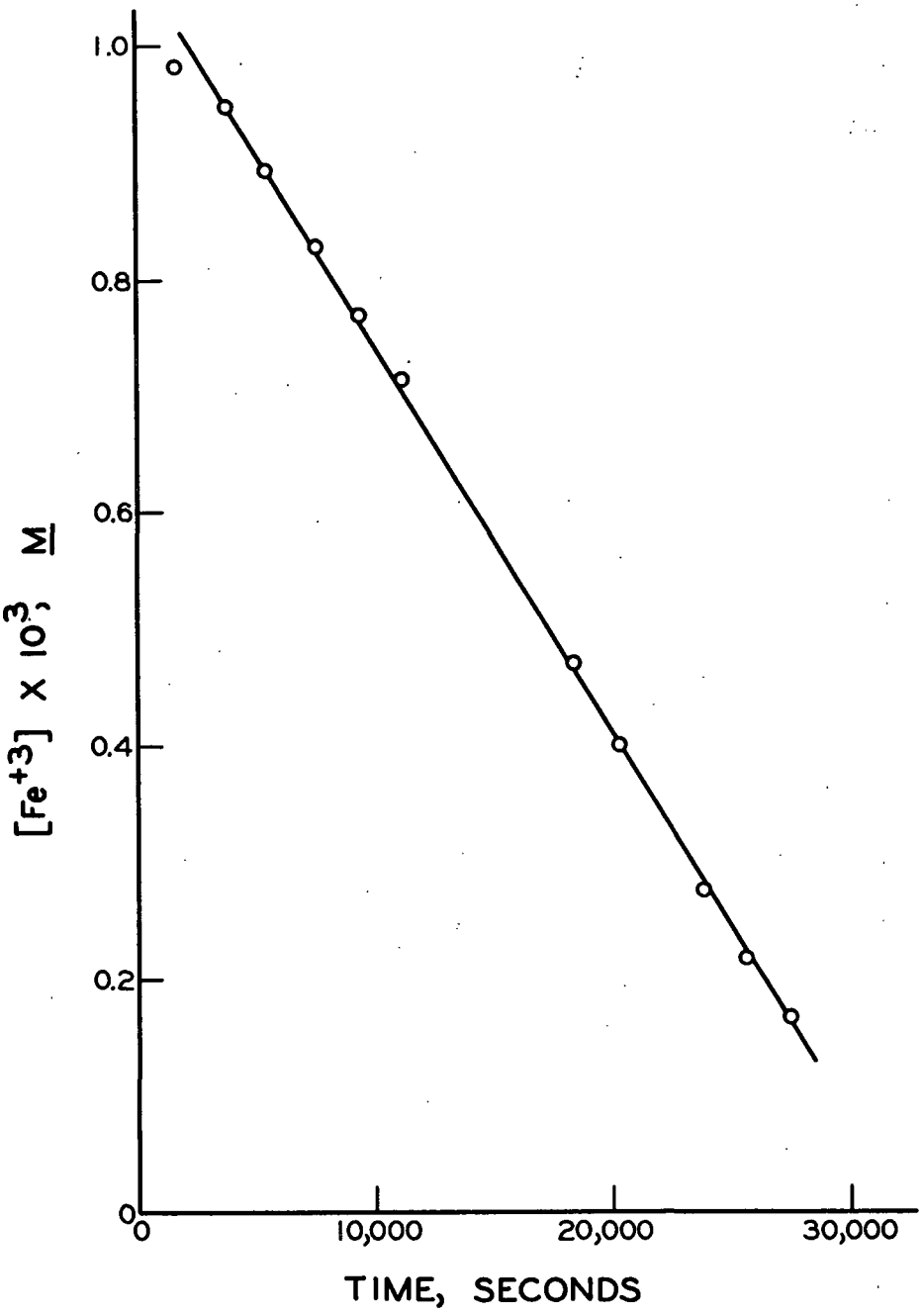


Figure 11. Pseudo-Zero-Order Plot for the Oxidation of 0.100M Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 1.00M $HClO_4$ at 88.6°C.

to follow this reaction. The extrapolated reaction curve does not pass through the origin of the plot because of a short warm-up period after placing the reaction ampoules in the constant-temperature bath.)

Figure 12 shows the effect of varying the glucose concentration. The pseudo-zero-order rate constant, k' is a function of the glucose concentration, the function assumed to be of the form of Equation (6). Converting Equation (6) to logarithmic form gives

$$\log k' = \log k + n \log [\text{Substrate}], \qquad (7)$$

which predicts that a plot of log k' against log [Substrate] will be a straight line with a slope equal to n. This plot is given in Fig. 13 for oxidations of glucose in 1M $HClO_4$ at 88.6°C. A straight line is obtained with a slope of 0.88.

In the preceding section, it was hypothesized that reduction of ferric ion by glucose solutions in high acidity media involves reaction between ferric ion and dehydration products of glucose. The kinetics of glucose oxidations in 1M $HClO_4$ are consistent with a mechanism involving dehydration of glucose in the rate-controlling step. Zero-order kinetics for ferric ion signify that the oxidant is not involved in the rate-determining step. This would be the case if this step were dehydration of glucose. The 0.88 order of reaction for glucose is also consistent with a dehydration mechanism, since Webb (50), in a study of the heat-induced breakdown of aqueous solutions of glucose, found that the rate of degradation was somewhat less than directly proportional to the glucose concentration.

Oxidations in 0.01M $HClO_4$.

Figure 9 shows that, at low acidities, rates of reaction are not constant (pseudo-zero-order kinetics) but decrease as reaction progresses. The kinetics

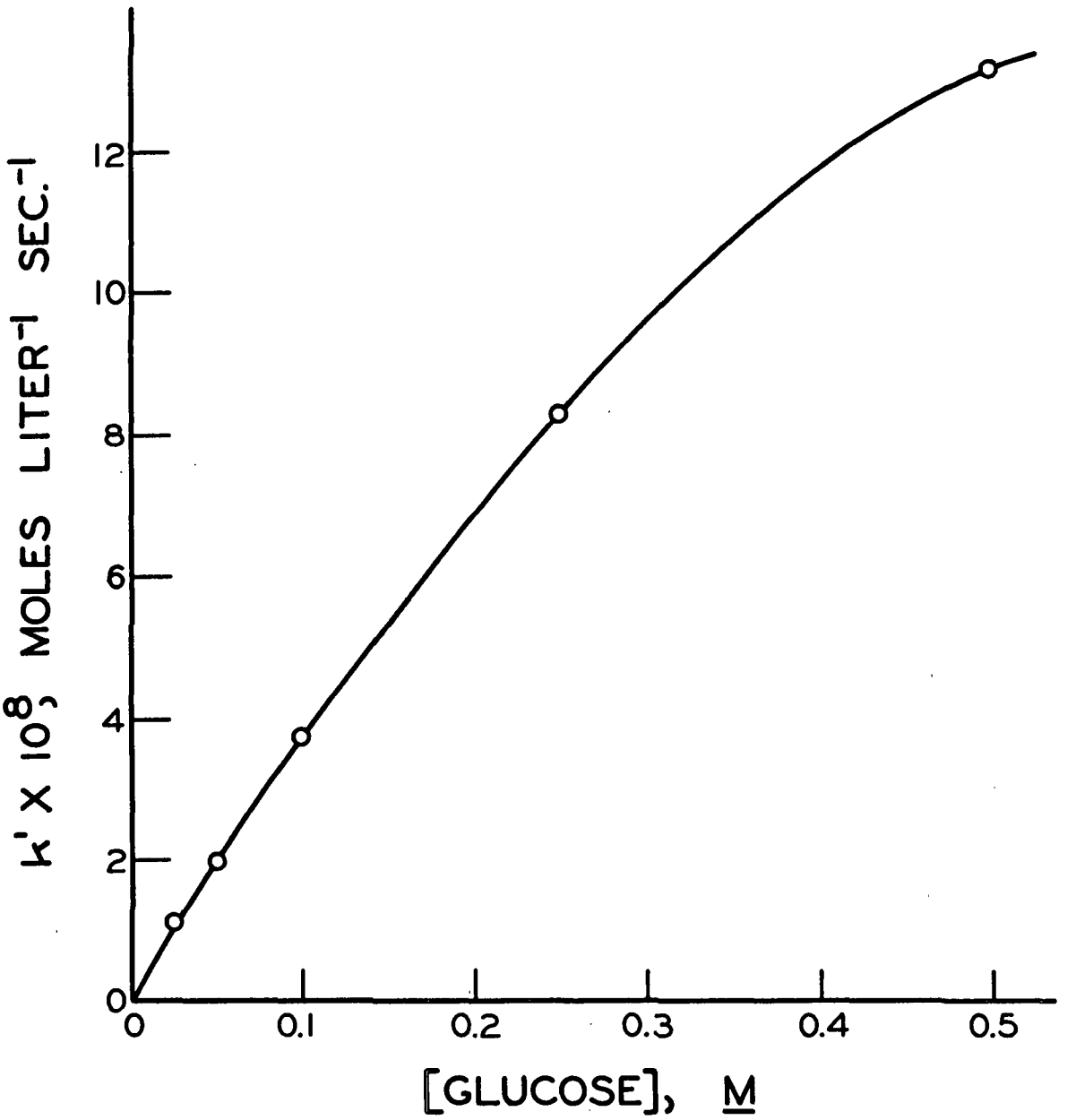


Figure 12. The Relationship Between Substrate Concentration and Reaction Rate Constant for the Oxidation of Glucose by 10^{-3} M $Fe(ClO_4)_3$ in 1.00 M $HClO_4$ at 88.6°C.

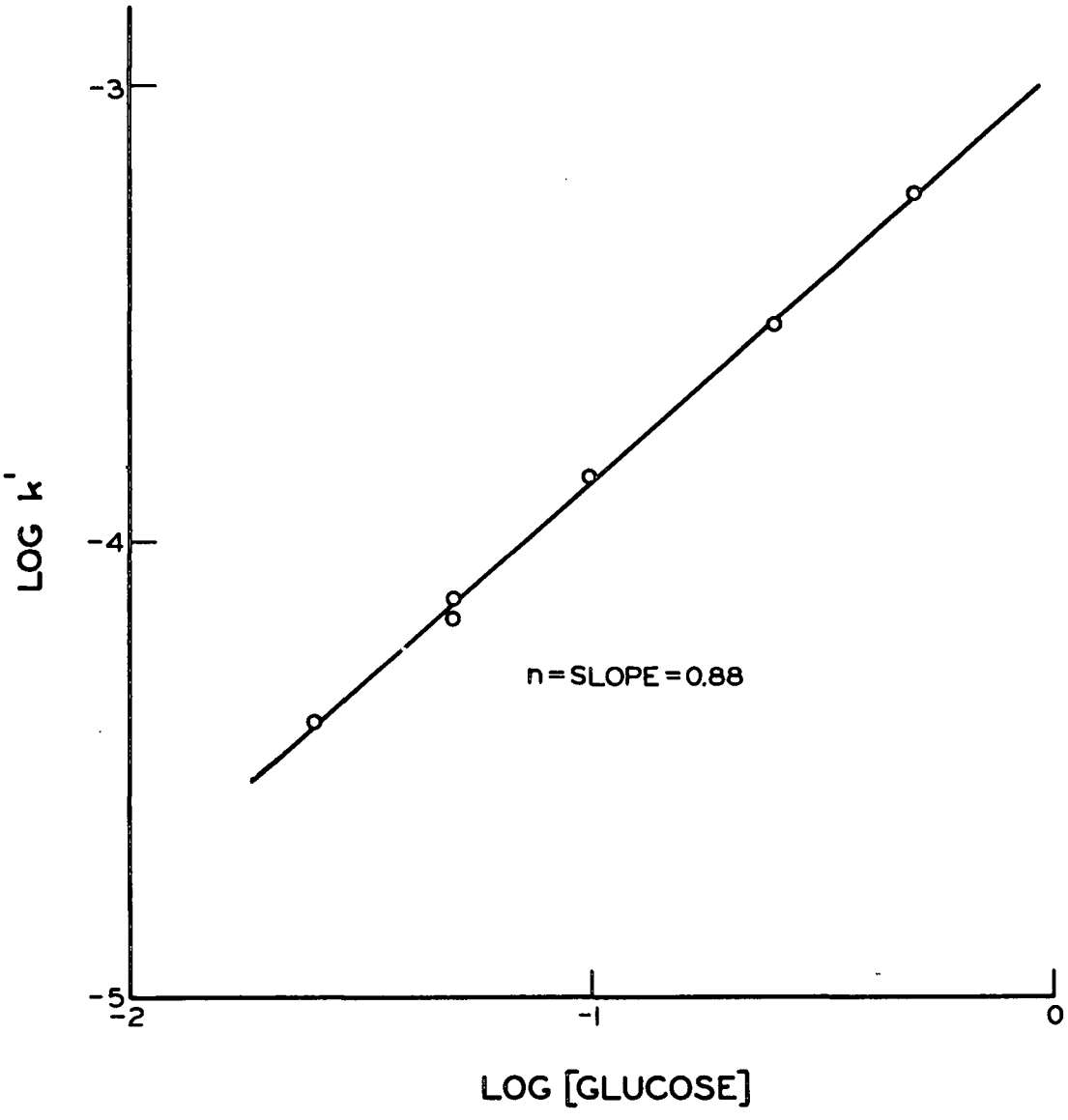


Figure 13. Log k' vs. Log[Glucose] Plot for the Oxidation of Glucose by 10^{-3} M $Fe(ClO_4)_3$ in 1.00M $HClO_4$ at 88.6°C.

of low acidity oxidations were studied in 0.01M $HClO_4$, where dehydration was shown to be unimportant by the following experiment.

Glucose (0.20M) was heated at 88.6°C. for 10 hours in a solution 0.01M $HClO_4$ and 0.99M in $NaClO_4$. At the end of this time, the solution was still water-white, indicating a lack of glucose dehydration. No precipitate formed when the solution was treated with 2,4-dinitrophenylhydrazine, showing that little or no dehydration of glucose took place under the conditions of the experiment.

In 0.01M $HClO_4$, the kinetics of glucose oxidations do not fit any one simple rate expression over the entire course of the reaction. However, plots of ln $[Fe^{+3}]$ against time gave straight lines (pseudo-first-order kinetics) after reaction had progressed beyond an initial induction period. (See Fig. 14.)

This type of behavior was encountered previously by Buist and Bunton (51) in the oxidation of 2-methylbutane-2,3-diol by periodic acid. They attributed the induction period to an initial slow build-up of an intermediate substrate-oxidant complex. This type of explanation was considered for the oxidation of glucose by ferric ion, but was rejected because the complexing study showed that equilibrium is reached very rapidly (< 30 sec.) in the glucose-ferric ion complexing reaction.

The possibility was also considered that the initial reaction does not follow pseudo-zero-order kinetics because of consecutive enolization and oxidation reactions of comparable rate during this period, as in oxidations of glycolaldehyde under certain conditions. (See pages 87-103.) But increasing the initial ferric ion concentration by a factor of ten did not cause the reaction to become pseudo-zero-order as would be the case if this explanation were correct.

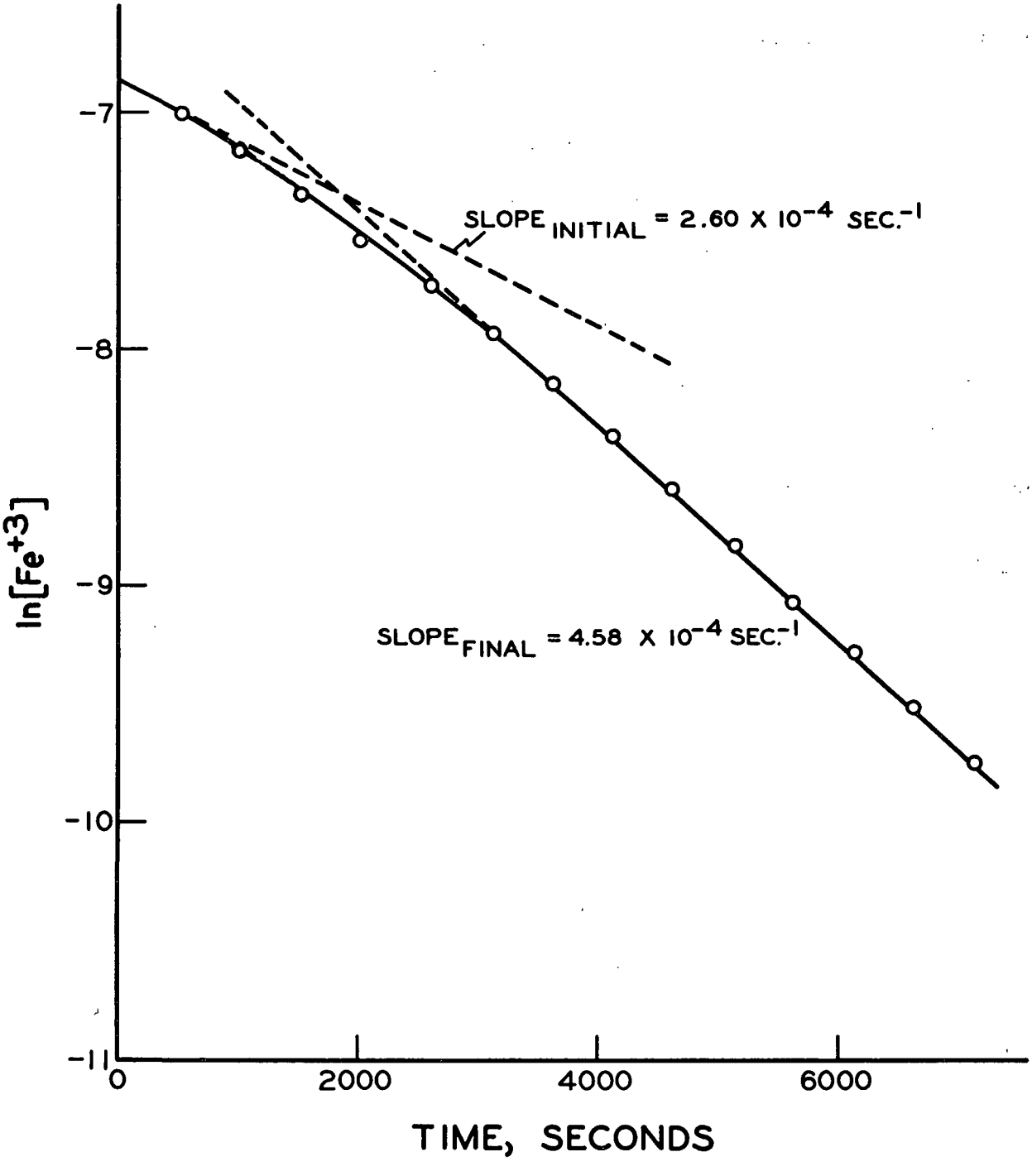


Figure 14. Pseudo-First-Order Plot for the Oxidation of 0.400M Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 90.0°C.

Based upon product analysis results (pages 52-62), it seems probable that the induction period is due to a build-up of an initial product which is oxidized rather easily by ferric ion.

Consider the reaction scheme,

$$G + Fe^{+3} \xrightarrow{k_1} F_G + Fe^{+2} + H^+$$

$$F_G + Fe^{+3} \xrightarrow{\text{fast}} P + Fe^{+2} + H^+$$

$$P + Fe^{+3} \xrightarrow{k_2} F_P + Fe^{+2} + H^+$$

$$F_P + Fe^{+3} \xrightarrow{\text{fast}} P' + Fe^{+2} + H,$$

where

$\underline{G}$ = glucose,

$\underline{F_G}$ = a free radical produced from glucose,

$\underline{P}$ = a product formed by oxidation of $\underline{F_G}$,

$\underline{F_P}$ = a free radical produced from $\underline{P}$, and

$\underline{P'}$ = product(s) formed by oxidation of $\underline{F_P}$.

The rate of reduction of ferric ion in the system is given by the equation,

$$-d[Fe^{+3}]/dt = 2k_1[G][Fe^{+3}] + 2k_2[P][Fe^{+3}] \qquad (8)$$

Since glucose is present in great excess, Equation (1) may be rewritten as

$$-d[Fe^{+3}]/dt = 2(k' + k_2[P])\,[Fe^{+3}], \qquad (9)$$

where $\underline{k'} = \underline{k_1}[\underline{G}]$.

The rates of formation and destruction of P are given by the equations

$$\text{rate formation} = k' [Fe^{+3}] \tag{10}$$

and

$$\text{rate destruction} = k [P][Fe^{+3}]. \tag{11}$$

During the early stages of reaction, the rate of formation of P exceeds the rate of destruction because [P] is small. Thus, the concentration of P builds up, and, at some point in time the condition will be reached where

$$k_2[P] = k' \tag{12}$$

(as long as $[Fe^{+3}]$ initial is sufficiently large to allow this to happen before all the ferric ion is consumed).

At this time, the rates of formation and destruction of P [Equations (10) and (11)] are equal and will remain equal thereafter (a consequence of the reaction being run in the presence of a large excess of glucose). Once the rate of destruction of P equals its rate of formation, there is no further build-up (or depletion) in the concentration of P. The constant or limiting concentration of P which is eventually attained is found from Equations (10) and (11) or from Equation (12) to be

$$[P]_{lim} = k'/k_2. \tag{13}$$

Substituting into Equation (9), one obtains

$$-d[Fe^{+3}]/dt = 4k'[Fe^{+3}] \tag{14}$$

for the rate of reaction once the limiting concentration of P is reached. This is the equation of a pseudo-first-order reaction, so pseudo-first-order kinetics should be followed during later reaction stages.

From Equation (9), the initial rate of reaction is $2\underline{k}'[Fe^{+3}]$, and, from Equation (11), the rate becomes $4\underline{k}[Fe^{+3}]$ once the limiting concentration of $\underline{P}_{\underline{P}}$ is attained. Therefore, the ratio of the slope of the linear portion of a first-order plot to the initial slope is $4\underline{k}'/2\underline{k}' = 2$.

The oxidation of glucose shown in Fig. 14 has a kinetic behavior similar to that of the system just described. The first-order plot initially has a continually increasing slope, which eventually becomes constant. Estimates of the final and initial slopes of this plot are 4.58×10^{-4} sec.$^{-1}$ and 2.60×10^{-4} sec.$^{-1}$, respectively. Their ratio is 1.8, close to the value of 2 predicted for a system of consecutive, competing reactions. Thus, it seems probable that the induction period in glucose oxidations is due to build-up of a reactive product.

Figures 15 and 16 show the effect that varying the glucose concentration has on the pseudo-first-order rate constant in 0.010M $HClO_4$ at 90.0°C. In Fig. 16, the pseudo-first-order rate constants obtained from the linear portions of first-order plots are shown as a function of glucose concentration. The relationship between rate and glucose concentration can be represented about equally well as a linear (first-order) or a curvilinear function. However, interpolation of the linear function to a glucose concentration of zero gives a significant positive intercept on the rate constant axis. Since blank reactions have shown that the rate of reduction of ferric ion is practically zero in the absence of a substrate, the true relationship between reaction rate and glucose concentration is probably curvilinear.

Duke (52) has analyzed the kinetics of oxidations involving breakdown of an intermediate substrate-oxidant complex in the rate-controlling step:

$$\text{Substrate} + \text{Oxidant} \underset{\text{fast}}{\overset{K}{\rightleftharpoons}} \text{Complex} \xrightarrow{k} \text{Products.}$$

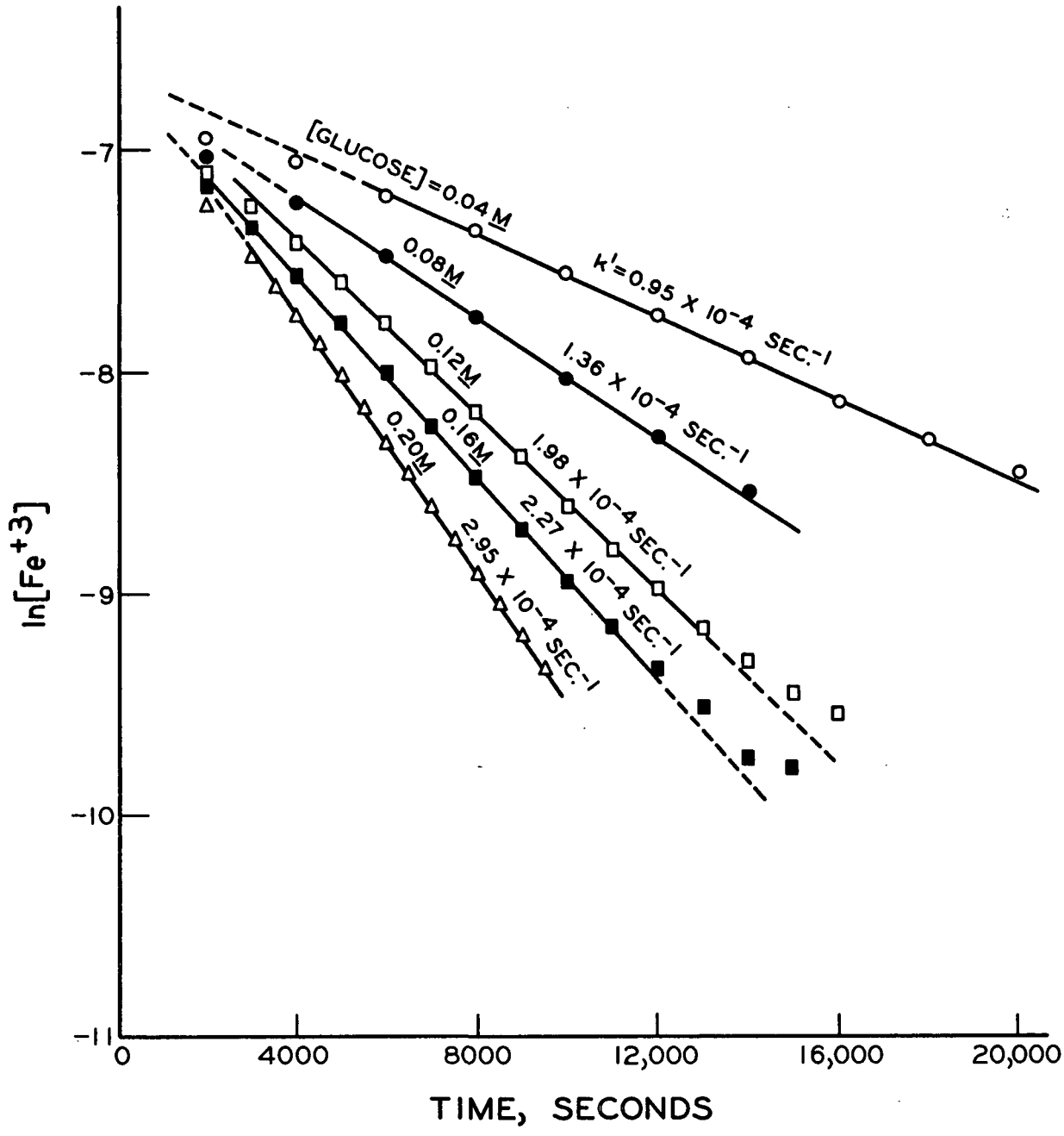


Figure 15. The Effect of Substrate Concentration on the Oxidation of Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 90.0°C.

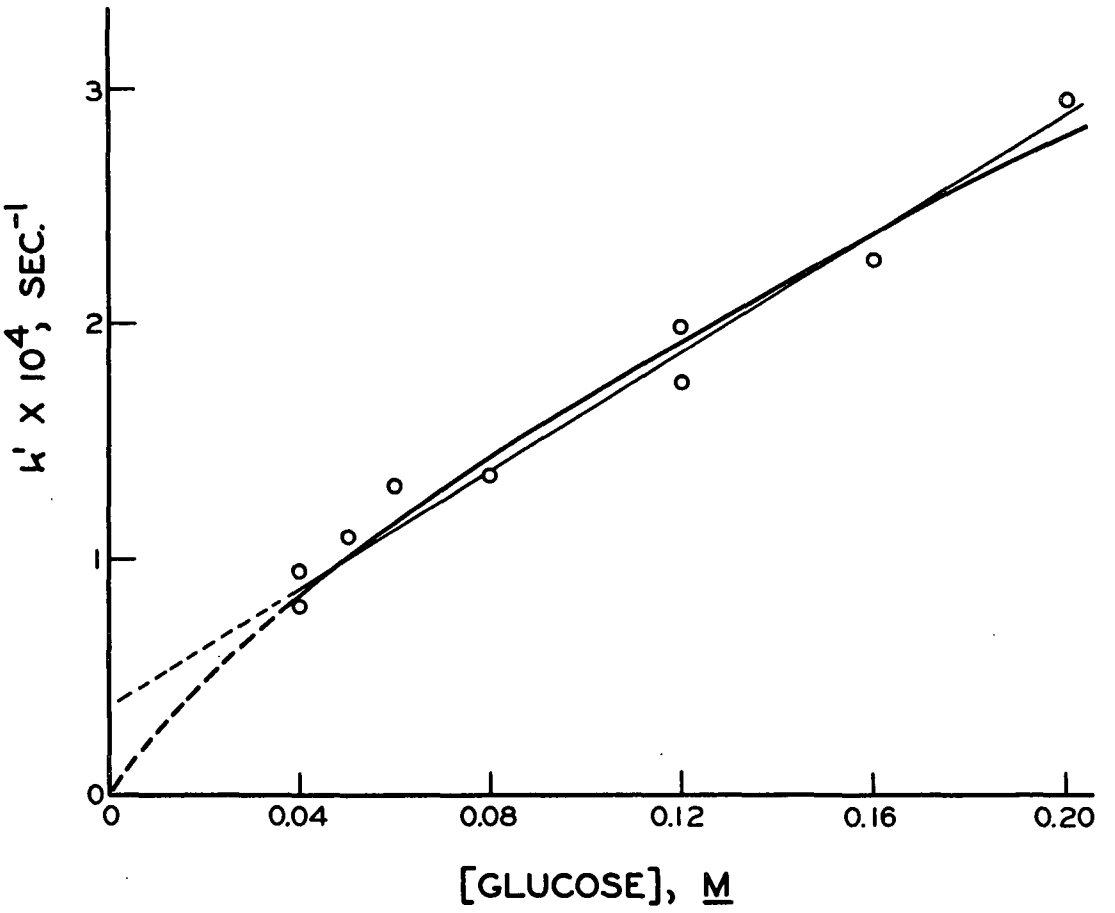


Figure 16. The Relationship Between the Substrate Concentration and the Reaction Rate Constant for the Oxidation of Glucose by 10^{-3} M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 90.0°C.

It was found that, when reactions are run with the substrate in excess, the relationship between the pseudo-first-order rate constant, k' and the substrate concentration [S], is given by the equation,

$$k' = kK[S]/(1 + K[S]), \qquad (15)$$

where k is the complex disproportionation rate constant, and K is the complex formation equilibrium constant. Inversion of Equation (15) gives

$$1/k' = 1/k + 1/kK[S], \qquad (16)$$

which predicts that a plot of $1/k'$ versus $1/[S]$ (Duke's reciprocal plot) will be a straight line.

This plot is shown in Fig. 17 for oxidations of glucose by ferric perchlorate. Although there is considerable scatter, the points fit a straight line reasonably well. Therefore, the dependence of reaction rate upon glucose concentration is consistent with a mechanism involving complex formation.

Temperature Dependence

Oxidations in 1M $HClO_4$

The effect of temperature upon the rate of oxidation of glucose by ferric perchlorate in 1M $HClO_4$ is shown by Fig. 18. The Arrhenius plot (Fig. 19) does not yield a straight line, indicating a complex reaction with a changing rate-controlling step. This is probably related to Webb's finding (50) that extrapolation of the Arrhenius equation from high to low temperature does not give a sensible estimate of the rate of dehydration of glucose solutions at room temperature.

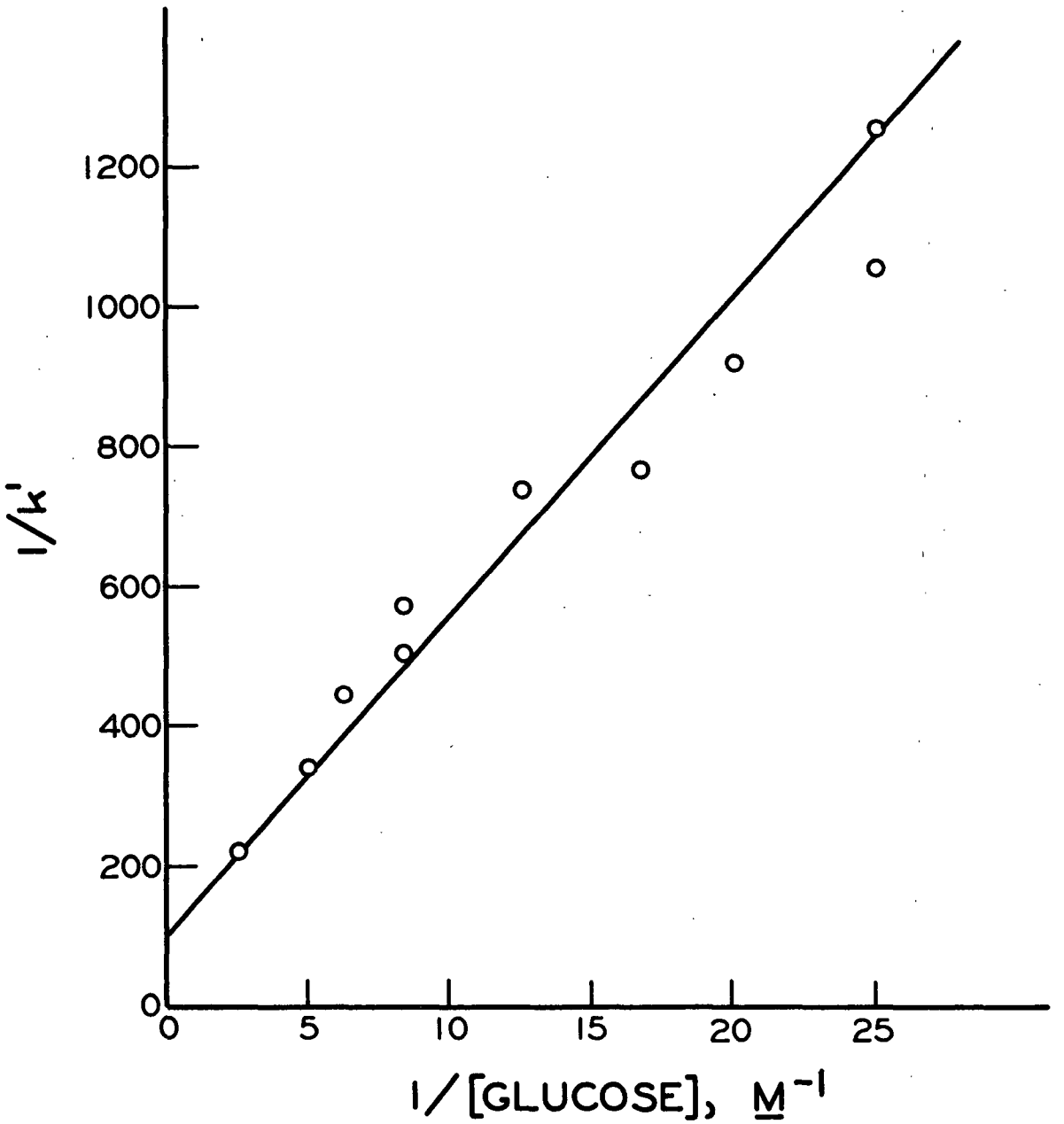


Figure 17. Duke's Reciprocal Plot for the Oxidation of Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 90.0°C.

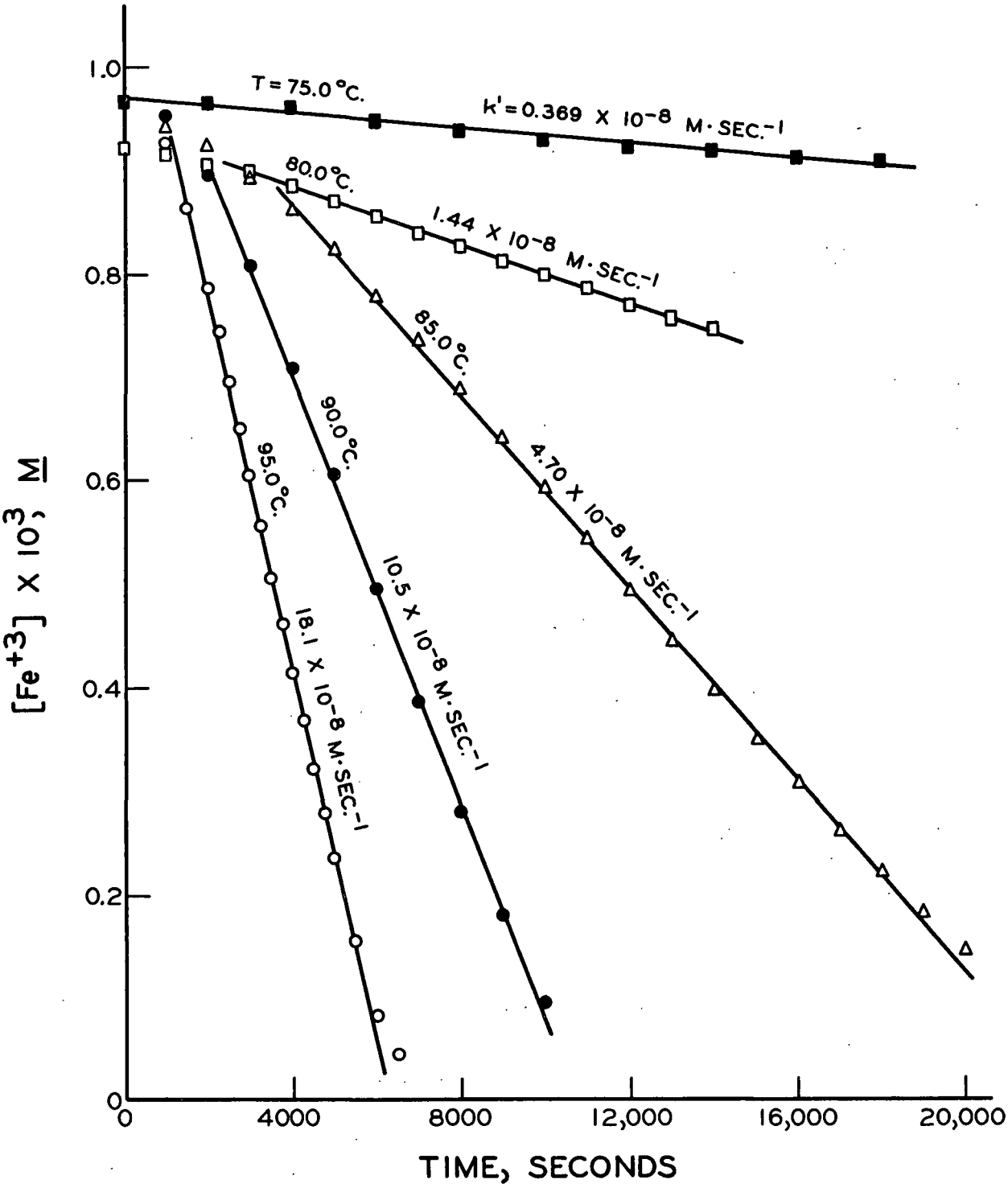


Figure 18. The Effect of Temperature on the Oxidation of 0.400M Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 1.00M $HClO_4$

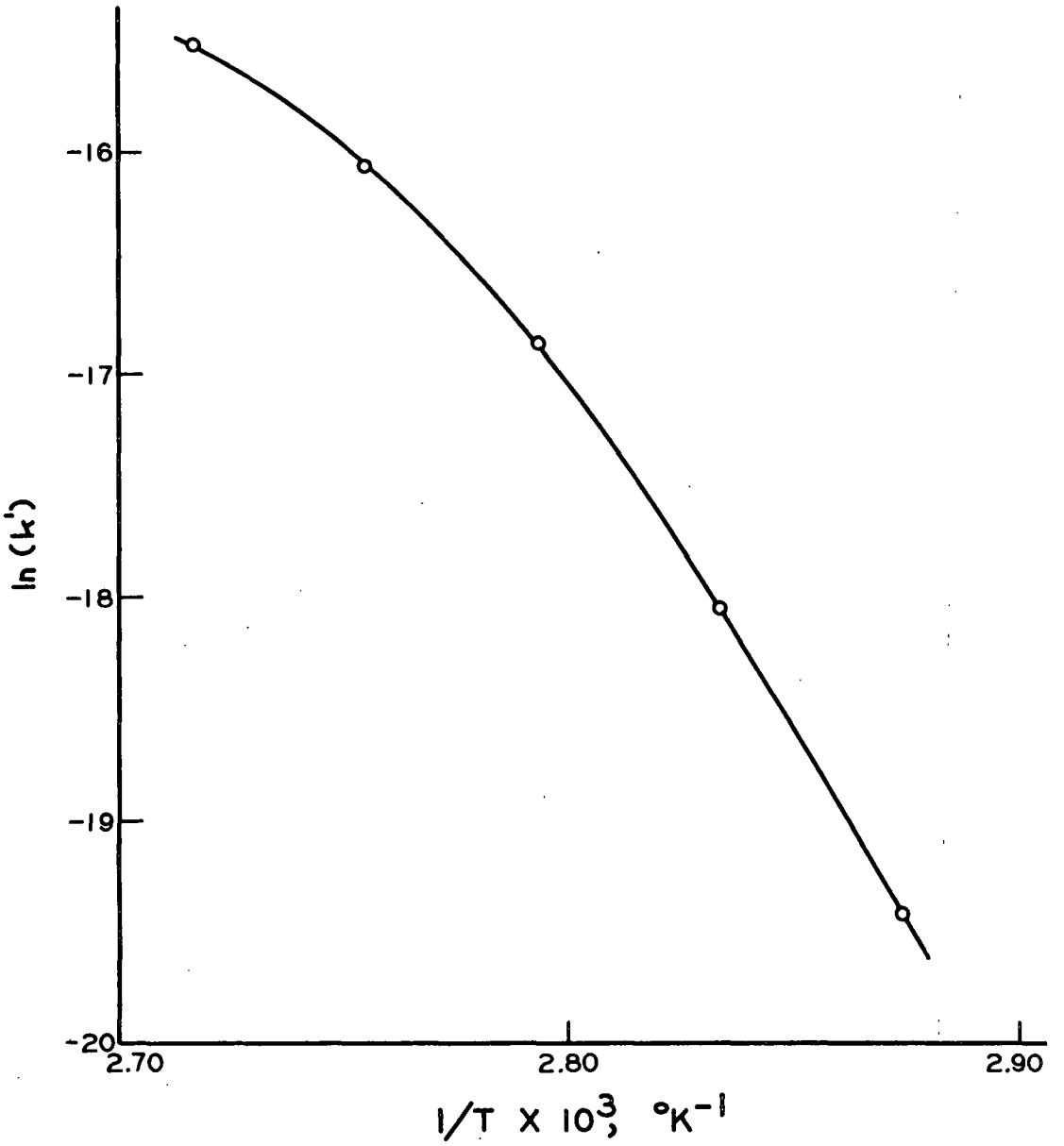


Figure 19. The Arrhenius Plot for the Oxidation of 0.400M Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 1.00M $HClO_4$

Oxidations in 0.01M $HClO_4$

Figure 20 shows the effect that temperature has on oxidations of glucose in 0.01M $HClO_4$. The Arrhenius rate law is followed in 0.01M $HClO_4$, and the activation energy calculated from the slope of the Arrhenius plot (Fig. 21) is 42.3 kcal./mole. This is considerably higher than the activation energy of 24.7 kcal./mole reported for the ferric ion oxidation of acetoin (15).

PRODUCT ANALYSIS

Qualitative Determination of Reaction Products

The products of ferric ion oxidation of glucose in 0.01M perchloric acid have been studied by derivatizing them with 2,4-dinitrophenylhydrazine followed by isolation and thin-layer chromatography of the 2,4-dinitrophenylhydrazine derivatives (DNPH's). This method has the advantage of separating the very small amounts of products from large amounts of unreacted glucose, since the latter reacts only very slowly with 2,4-dinitrophenylhydrazine at room temperature.

Peifer's microchromatoplate technique (53) was used for chromatographic comparison of reaction mixture DNPH's with those of known compounds. In this method, DNPH's dissolved in tetrahydrofuran are spotted onto microscope slides coated with a very thin layer of silica gel. Development is rapid, and excellent separations of complex mixtures of DNPH's can be achieved even though the development distance is only 5 cm. Apparently, the extreme thinness of the layers accounts for the great resolving power of microchromatoplates.

The one disadvantage of this technique, that R_F values are not reproducible from plate to plate (probably because of varying layer thicknesses), is offset by the finding that ratios of R_F values,

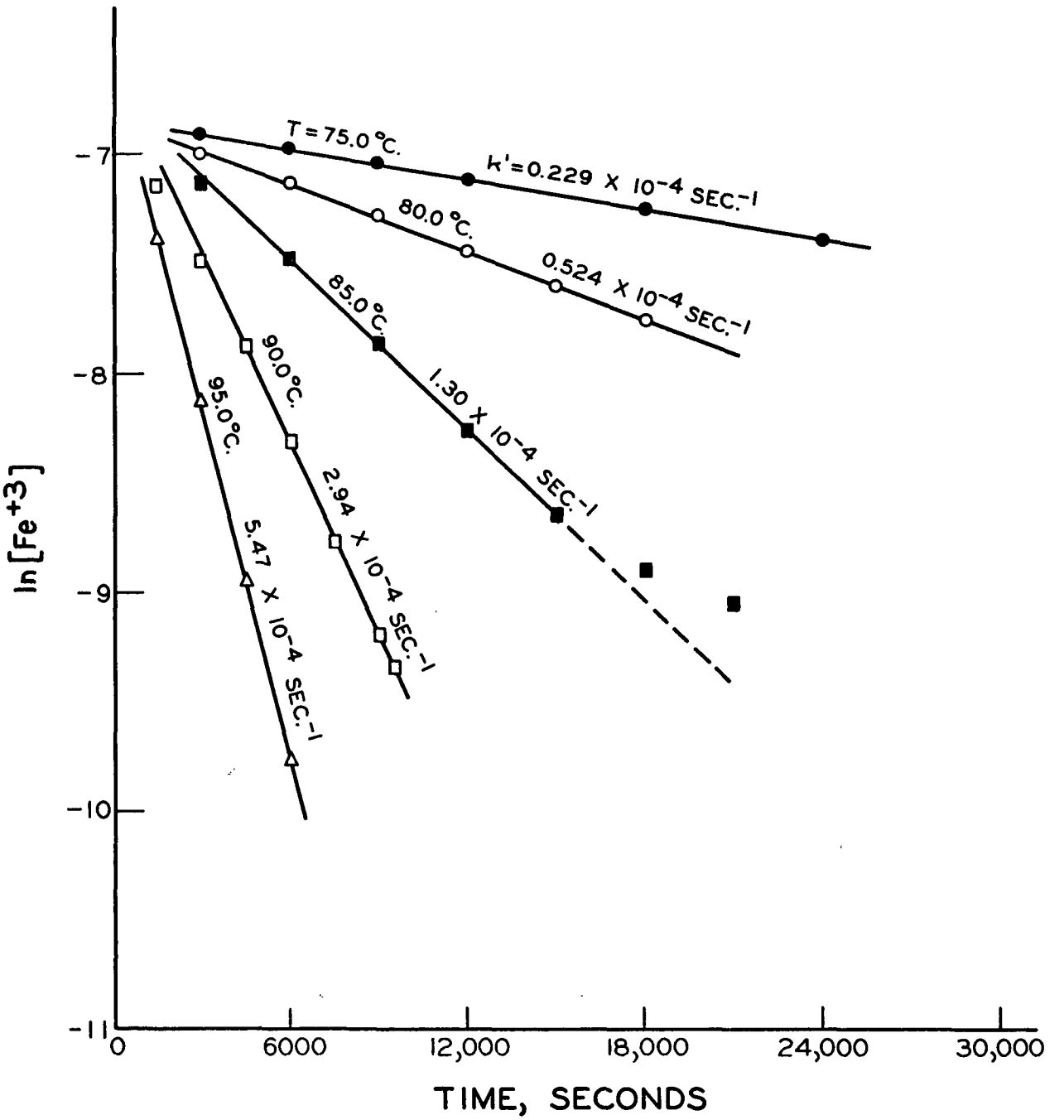


Figure 20. The Effect of Temperature on the Oxidation of 0.200M Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$

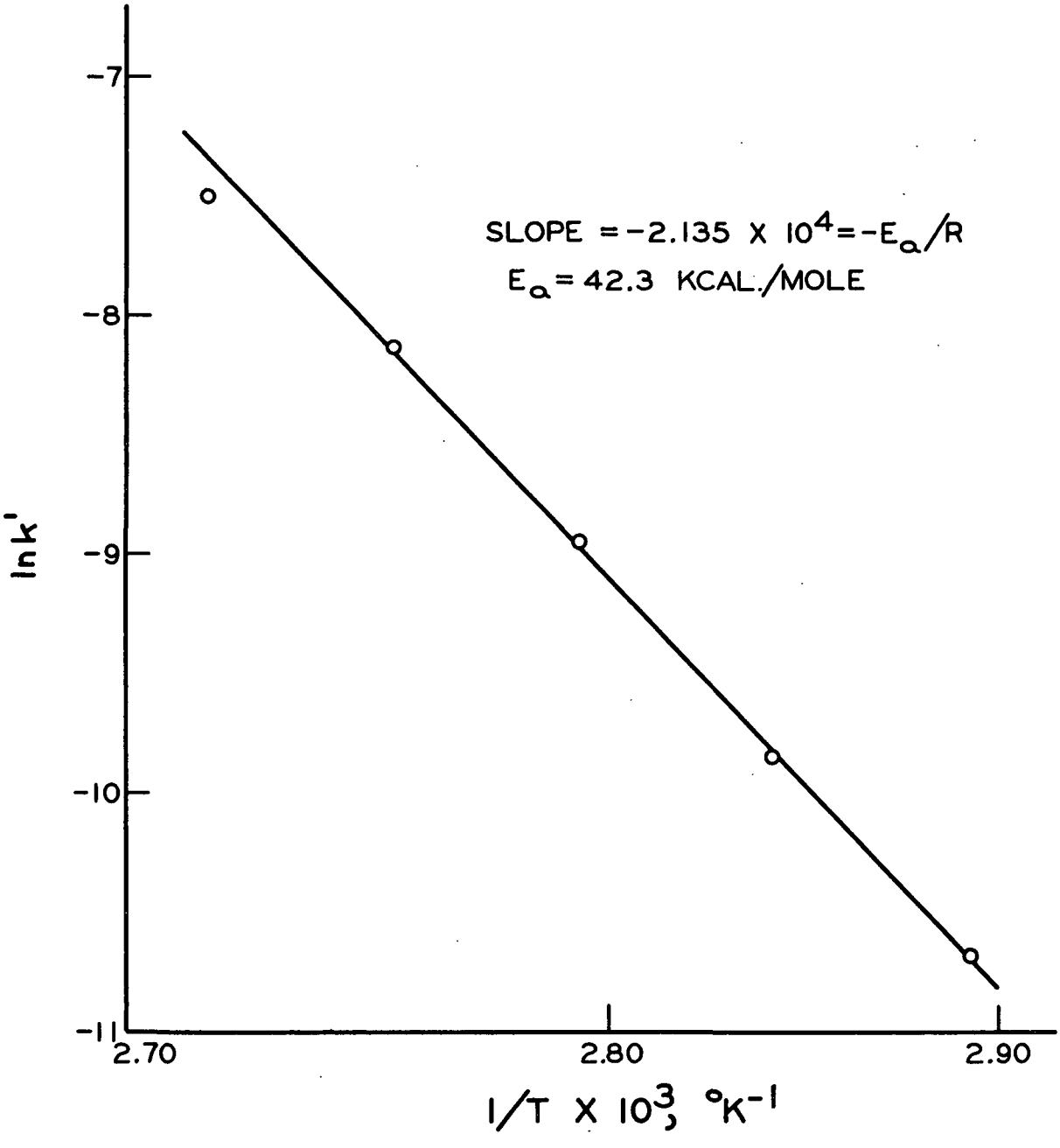


Figure 21. The Arrhenius Plot for the Oxidation of 0.200M Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$

$$R_F \text{ DNPH 1} / R_F \text{ DNPH 2},$$

are reproducible from plate to plate and are the same as those obtained on standard-sized thin-layer plates. In this study all substances were compared with the fast-moving component of glyoxal bis-2,4-dinitrophenylhydrazone. [Glyoxal bis-2,4-dinotrophenylhydrazone was found to contain two chromatographically-different components, probably syn- and antiforms (54).] Results were recorded as R_g values, defined by

$$R_g \text{ of substance A} = \frac{\text{distance moved by substance A}}{\text{distance moved by glyoxal DNPH (fast component)}}.$$

When precipitates from 2,4-dinitrophenylhydrazine-treated glucose oxidation mixtures were chromatographed in benzene/tetrahydrofuran (80:20) seven spots were distinguishable. Table II gives the R_g values of these DNPH's (determined after separation from one another by preparative thin-layer chromatography). A number of DNPH's were prepared from known compounds and their R_g values are listed in Table III.

Byrne (55) studied the separation of DNPH's of products from pyrolysis of cotton cellulose. Thin-layer chromatography was carried out on 41 compounds, and distances travelled by them are given in his paper for a number of solvent systems, R_g values calculated from his data and from similar work by Anet (56) are listed in Table III for some possible oxidation products of glucose.

On the basis of R_g values, four of the oxidation product DNPH's can be tentatively identified. Glyoxal bis-2,4-dinitrophenylhydrazone (2 components), hydroxypyruvaldehyde bis-2,4-dinitrophenylhydrazone, and glucose 2,4-dinitrophenylosazone appear to be present in DNPH mixtures obtained from glucose reaction

TABLE II

THIN-LAYER CHROMATOGRAPHY OF GLUCOSE REACTION MIXTURE DNPH's

Component	R_g in Solvent A[a]	R_g in Solvent B[b]	R_g in Solvent C[c]	Order of Intensity[d]	Color[e]
1	1.00	1.00	1.00	1	Blue
2	0.89 ± .02[f](9)[g]	0.52 ± .03 (5)	0.73 (1)	3	Blue
3	0.76 ± .05 (4)	0.67 ± .05 (3)	-	3	Blue
4	0.50 ± .05 (8)	0.09 ± .00 (2)	-	4	Blue
5	0.20 ± .05 (4)	0.00 ± .00 (3)	-	4	Blue
6	0.08 ± .02 (4)	0.00 ± .00 (3)	-	2	Purple
7	0.00 ± .01 (3)	0.00 ± .00 (3)	-	5	Blue

[a]Solvent A = benzene tetrahydrofuran (80:20).

[b]Solvent B = benzene/tetrahydrofuran (93:7).

[c]Solvent C = toluene/ethyl acetate (3:1).

[d]The most intense spot is labeled 1, the least 5.

[e]Color after spraying with ethanolamine. A blue or purple coloration is characteristic of bis-DNPH's (55).

[f]95% Confidence interval.

TABLE III

THIN-LAYER CHROMATOGRAPHY OF KNOWN DNPH's

	$R_{glyoxal}$ in Solvent		
	Literature Values		
DNPH of	A (55)	B (55)	C (56)
Glyoxal (bis)	1.00	1.00	1.00
Glucose (bis)	0.05	0.01	0.00
Erythrose (bis)	0.81	0.39	--
Hydroxypyruvaldehyde (bis)	0.87	0.51	0.72
5-Hydroxymethylfurfural	0.45 & 0.48	0.18 & 0.27	--
Mesoxaldehyde (tris)	--	0.44	--
Mesoxaldehyde (bis)	--	0.65	--
Pyruvaldehyde (bis)	1.03	1.17	--
		Observed Values	
Glyoxal (bis)	1.00 & 0.76	1.00 & 0.67	--
Hydroxypyruvaldehyde (bis)	0.87	0.50	--
5-Hydroxymethylfurfural	0.44	0.19	--

Solvent A = benzene/tetrahydrofuran (80:20).

Solvent B = benzene/tetrahydrofuran (93:7).

Solvent C = toluene/ethyl acetate (3:1).

solutions. The remaining three DNPH's have chromatographic mobilities similar to those of three components obtained by heating glucose solutions with 2,4-dinitrophenylhydrazine. They may be products of side reactions between glucose and 2,4-dinitrophenylhydrazine or glucose and acid.

Column chromatography was used to separate the reaction product DNPH's for x-ray analysis. After column chromatography, two more DNPH's became noticeable. One had $\underline{R}_g$'s of 0.80 and 0.39, the other 1.05 and 1.18 in benzene/tetrahydrofuran (80:20) and (93:7) respectively. From their $\underline{R}_g$ values these components appear to be erythrose 2,4-dinitrophenylosazone and pyruvaldehyde bis-2,4-dinitrophenylhydrazone, respectively. It is not known whether these components were originally present but undetectable in the mixture of oxidation product DNPH's or whether they are artifacts produced during column chromatography.

X-ray diffraction patterns of the major reaction mixture DNPH's were compared with those from known compounds. Only glyoxal bis-DNPH could be definitely identified. The tentatively-identified glyoxal bis-DNPH from glucose oxidation contained only the fast-moving component after column chromatography, whereas glyoxal bis-DNPH prepared from glyoxal di(sodium bisulfite) contained both components listed in Table III. The diffraction pattern of the reaction mixture DNPH was identical with that of the known compound, confirming the identification based upon chromatographic behavior.

The diffraction patterns of the reaction mixture DNPH's tentatively identified as hydroxypyruvaldehyde bis-2,4-dinitrophenylhydrazone, erythrose 2,4-dinitrophenylosazone, and glucose 2,4-dinitrophenylosazone were compared with those of the known compounds. The diffraction lines in the patterns of these compounds were not clear enough to allow definite identifications to be made. However, the patterns of the corresponding known and reaction mixture DNPH's were very similar in all three cases.

Interpretation of product analysis results is hindered by the fact that several compounds give rise to the same bis-2,4-dinitrophenylhydrazone. This is a consequence of the ability of 2,4-dinitrophenylhydrazine to oxidize and derivatize hydroxyl groups adjacent to a carbonyl group. Thus, glyoxal and glycolaldehyde both form glyoxal bis-2,4-dinitrophenylhydrazone when treated with 2,4-dinitrophenylhydrazine in hydrochloric acid. The parent compound of hydroxypyruvaldehyde bis-2,4-dinitrophenylhydrazone could be glyceraldehyde, hydroxypyruvaldehyde (glycerosone), or dihydroxyacetone.

Pyruvaldehyde bis-2,4-dinitrophenylhydrazone could have been formed from pyruvaldehyde, 2-hydroxypropanal, or 2-hydroxypropanone. But it could also have been formed from glyceraldehyde, since hydroxymethyl group reduction has been reported (57) to occur when glyceraldehyde is derivatized with 2,4-dinitrophenylhydrazine.

Glucose 2,4-dinitrophenylosazone might have been formed from glucosone, glucose, fructose, or mannose. However, treatment of solutions of the latter three sugars with 2,4-dinitrophenylhydrazine for 6 hours at room temperature did not give rise to any visible DNPH. Since glucose reaction mixtures were generally treated for only one hour with 2,4-dinitrophenylhydrazine, it seems likely that glucose 2,4-dinitrophenylosazone was formed from glucosone, particularly since glucosone is known to react readily with 2,4-dinitrophenylhydrazine in the cold (58).

In conclusion, the product analysis results indicate the presence of two-, three-, and four-carbon carbonyl compounds in solutions of glucose which have been oxidized by ferric ion. They also suggest that it is highly likely that glucosone is an oxidation product.

QUANTITATIVE ANALYSIS OF REACTION PRODUCTS

A reaction mixture, of initial composition

$[Fe(ClO_4)_3] = 10.20 \times 10^{-4}$ M

[Glucose] = 0.10 M

$[HClO_4] = 0.01$M

$[NaClO_4] = 0.99$M,

was reacted at 90°C. for about 4.5 hours, during which time the ferric ion concentration fell to 0.22×10^{-4} M. After cooling, a 25.00-ml. aliquot of the reaction mixture was treated with 25.00 ml. of saturated 2,4-dinitrophenylhydrazine in 2M HCl for 6 hours at room temperature. The precipitate which was obtained weighed 3.7 mg. after drying. [Glucose (0.10M) gave no precipitate when treated with 2,4-dinitrophenylhydrazine under the same conditions.] From the amount of ferric ion consumed, a precipitate weighing 6.7 mg. would be obtained if glucose were oxidized quantitatively to glucosone.

The precipitate was dissolved in tetrahydrofuran, diluted to 10.00 ml., and 1.00-ml. portions were chromatographed by thin-layer chromatography with benzene-tetrahydrofuran mixtures. Zones were scraped off the plates and DNPH's were eluted from the silica gel with tetrahydrofuran. Absorbances of the various fractions were measured at 440 nm. after dilution to 10.00 ml. with tetrahydrofuran. Fractions which were not pure were rechromatographed until they contained only one component. From the absorbances which were measured after the isolation of each fraction, the absorbances due to glucose 2,4-dinitrophenylosazone and glyoxal bis-2,4-dinitrophenylhydrazone were obtained and are given in Table IV.

The molar absorbance of the latter compound is 44,000 liter · $mole^{-1}$ · $cm.^{-1}$ (59). That of the former compound was determined to be 34,200 liter · $mole^{-1}$ · $cm.^{-1}$ From the molar absorbance values and the absorbances of the DNPH's, it can be

TABLE IV

QUANTITATIVE ANALYSIS OF A MIXTURE OF 2,4-DINITROPHENYLHYDRAZINE DERIVATIVES FROM A GLUCOSE OXIDATION MIXTURE

Absorbance (at 440 nm.) of				
Fraction 1[a]	Fraction 2[b]	Fraction 3[c]	% of Absorbance of Fraction 3 Due to Glyoxal DNPH	Absorbance of Glyoxal DNPH
1.047	--	--	--	--
1.034	0.203	2.260	89.7	2.025
1.072	0.202	2.255	90.0	2.030
1.005	0.237	2.270	88.6	2.010
0.982	0.237	2.215	90.6	2.005
0.972	0.229	2.270	92.0	2.085
1.019				2.030

[a]Contains the component thought to be glucose 2,4-dinitrophenylosazone.

[b]Consists primarily of two unidentified DNPH's which are also formed when glucose is heated with 2,4-dinitrophenylhydrazine.

[c]Contains mainly glyoxal bis-2,4-dinitrophenylhydrazone plus a small amount of the DNPH thought to be hydroxypyruvaldehyde bis-2,4-dinitrophenylhydrazone.

calculated that the concentrations of glucosone* and glyoxal in the reaction mixture are 1.19×10^{-4} M and 1.84×10^{-4} M, respectively.

The change in ferric ion concentration was 9.98×10^{-4} M. Assuming that two moles of ferric ion are consumed per mole of glucosone formed and that the remainder of the ferric ion is used to form glyoxal, the change in ferric ion concentration due to glyoxal formation is $(9.98 - 2.38) \times 10^{-4}$ M $= 7.60 \times 10^{-4}$ M. Therefore, the number of moles of ferric ion consumed per mole of glyoxal formed is estimated to be 7.60×10^{-4} M/1.84×10^{-4} M $= 4.12$, or about 4.

Since all the ferric ion is accounted for by a reasonable stoichiometry for the observed products, other possible products, such as gluconic acid are probably not present in significant amounts. Other products were not detected by paper chromatography, but this could have been due to insufficient sensitivity.

PROPOSED REACTION MECHANISM

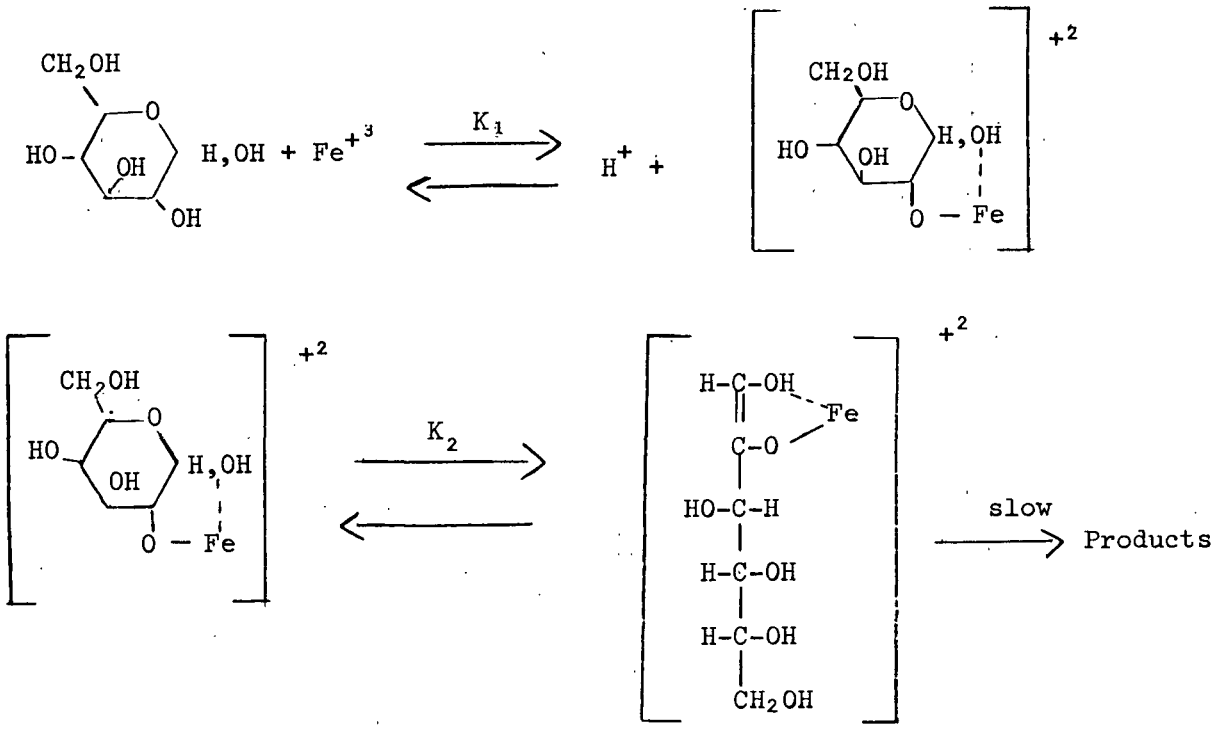
The initial oxidation of glucose is believed to occur as proposed by Kraske (11), by complex formation between ferric ion and the 1,2-enediol of glucose. Although no direct evidence was obtained for oxidation of glucose via the enediol,

*For convenience' sake, the quantitative analysis discussion is being conducted as though glucosone and glyoxal are the reaction products which give rise to the observed DNPH's.

the latter is assumed to be the reactive organic species* since enolization appears to be involved in the oxidation of the α-hydroxyaldehyde grouping of glycolaldehyde (see p. 87-103) and since ferric ion oxidations of stable enediols are known to involve complex formation (12).

Product analysis results indicate that glyoxal (or glycolaldehyde) and glucosone are the major carbonyl products of glucose oxidations by ferric perchlorate. Evidence has also been obtained for the presence of a significant amount of a three-carbon fragment and a small amount of a four-carbon fragment in reaction mixtures.

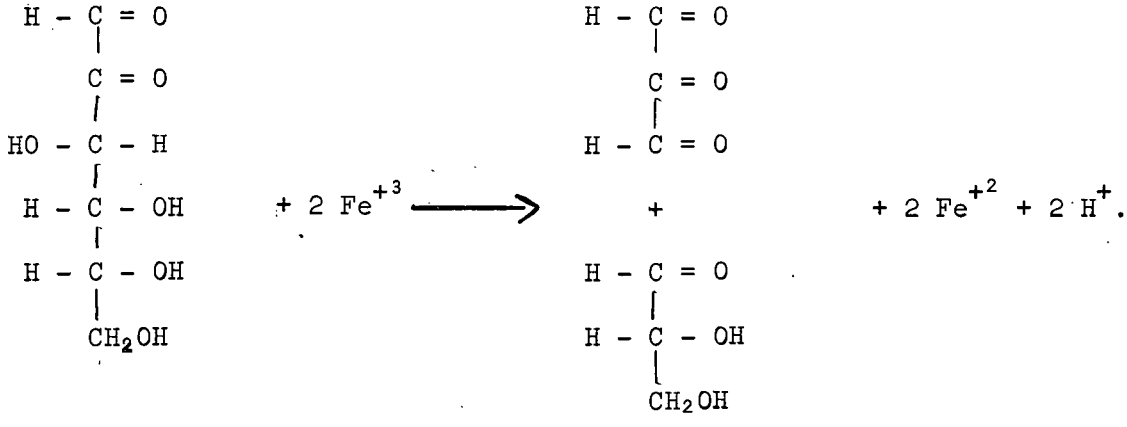
*The following alternate mechanism cannot be excluded:



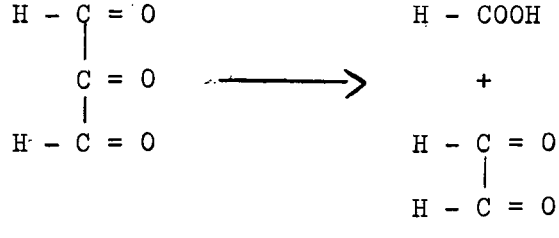
But this mechanism is not very different from the one proposed by Kraske since the actual oxidation step involves breakdown of a ferric ion-enediol complex. The main difference is that the alternate mechanism assumes that the ferric ion-enediol complex is formed from a complex between ferric ion and the hemiacetal structure of glucose.

Both kinetics and products of glucose oxidations are in accord with a reaction scheme involving oxidation of glucose to glucosone, followed by oxidation and cleavage of glucosone. The way in which the carbon-carbon bond cleavage is brought about is somewhat uncertain. It could take place either as a result of oxidative cleavage of glucosone or by breakdown of an unstable oxidation product of glucosone.

Considering the former alternative, oxidative cleavage of the C-3 — C-4 bond of glucosone might give rise to mesoxaldehyde and glyceraldehyde as shown below.

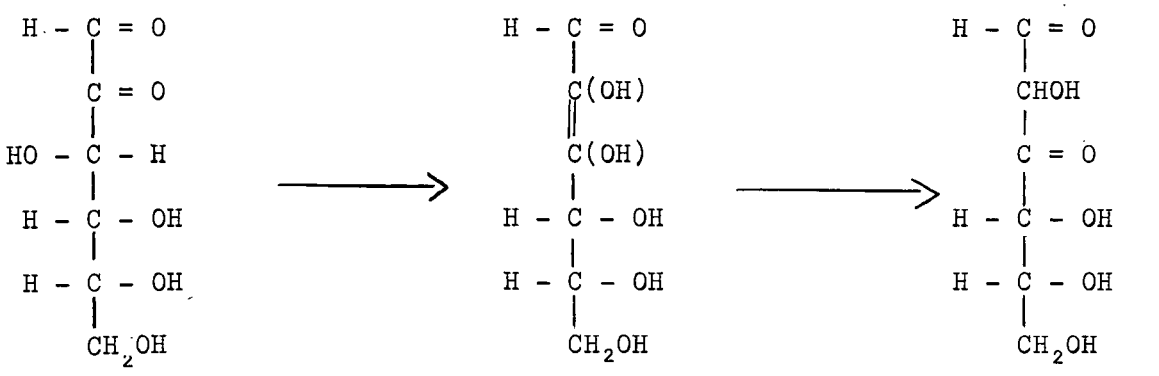


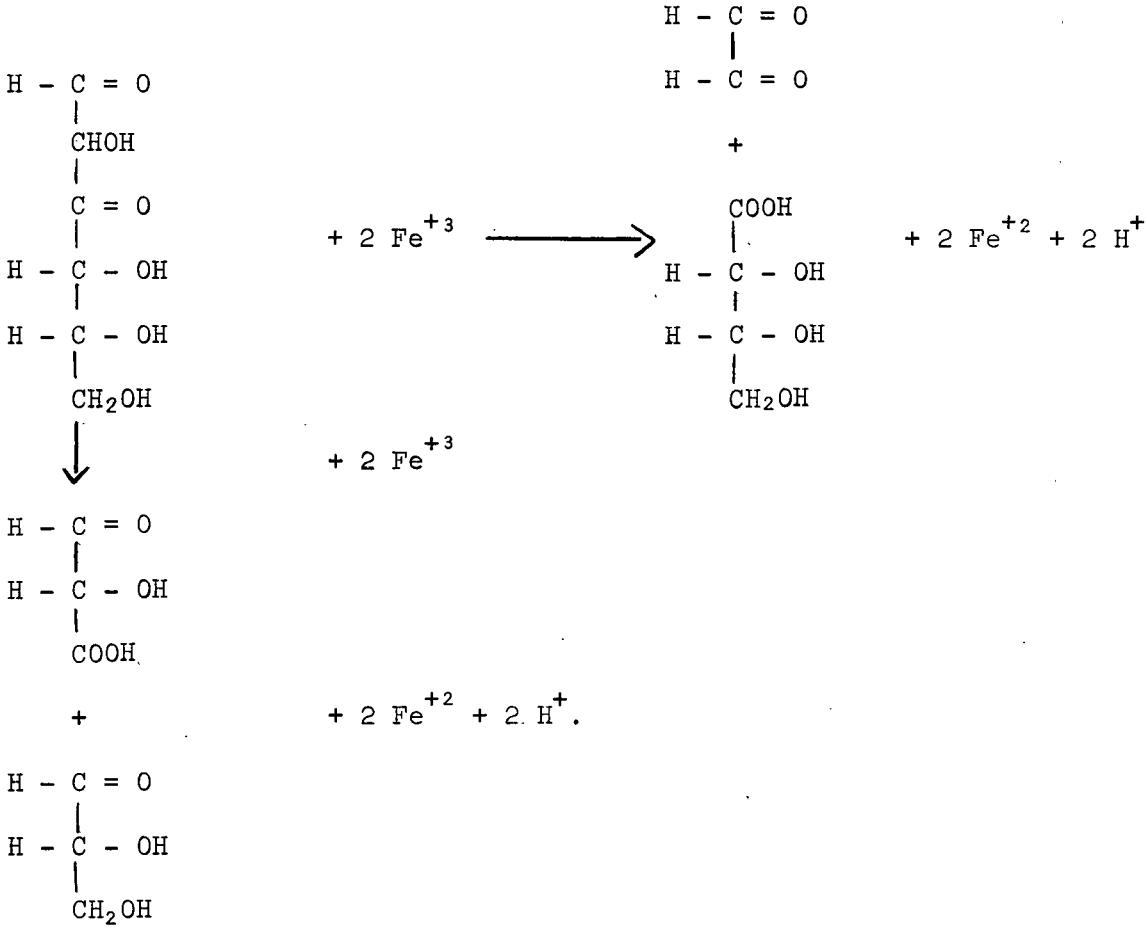
There is reason to think that mesoxaldehyde might be unstable under reaction conditions (see p. 67) and might break down to give glyoxal and formic acid.



This scheme would explain the presence of two- and three-carbon compounds in glucose reaction mixtures, but from the apparent specificity of ferric ion for the α-hydroxycarbonyl grouping, it seems unlikely that the C-3 — C-4 bond would be attacked by ferric ion in preference to the C-2 — C-3 α-hydroxycarbonyl grouping.

Oxidative cleavage of a 3-keto sugar formed by isomerization of glucosone (via enolization) would give rise to the observed products and stoichiometry:





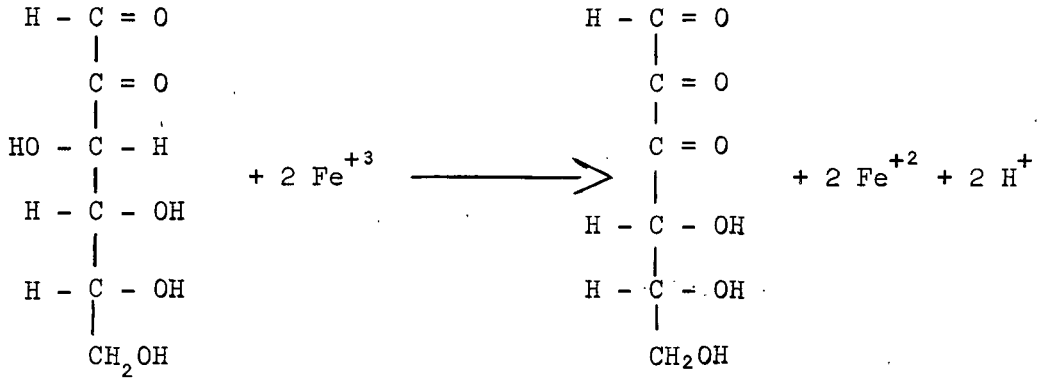
The acid resulting from oxidative cleavage of the C-3 — C-4 bond, 2-hydroxy-3-oxo-propionic acid, has been reported to undergo decarboxylation when heated with

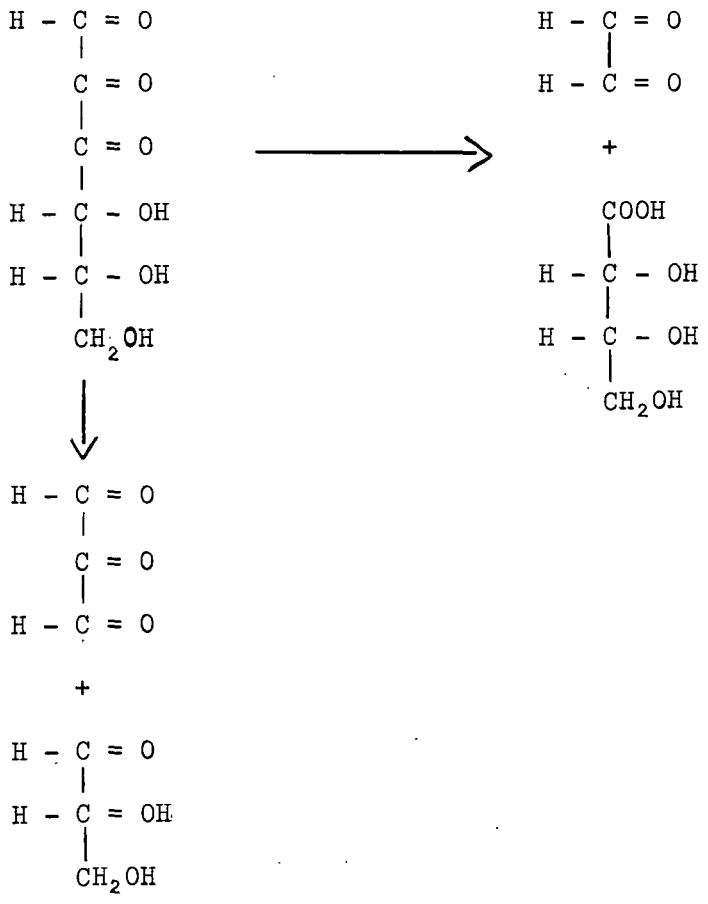
phenylhydrazine in an acid solution, forming glyoxal bis-phenylhydrazone (60). A similar reaction in the present study might lead to the formation of glyoxal bis-2,4-dinitrophenylhydrazone.

This mechanism accounts for the presence of two- and three-carbon fragments in reaction mixtures and for the stoichiometry of 4 moles of ferric ion consumed per mole of glyoxal bis-2,4-dinitrophenylhydrazone formed. But there is no apparent reason why glucosone should have to isomerize to the 3-keto sugar before being oxidized by ferric ion. One would think that it could be readily attacked by ferric ion at the C-2 — C-3 α-hydroxycarbonyl grouping, but oxidative cleavage of the C-2 — C-3 bond would not give the observed products.

Russian researchers have shown that many carbon-carbon bond cleavages result from oxidations followed by subsequent nonoxidative cleavages of labile oxidation products (61). Oxidation promotes bond breakage by introducing into molecules polar substituents which can activate nearby carbon-carbon bonds to undergo hydrolysis. In many cases, the greater the degree of oxidation of a molecule, the more readily will cleavage occur. The hydrolysis of carbon-carbon bonds has been shown to be catalyzed by both acids and bases and to occur more readily at high than at low temperatures.

Oxidation of glucosone to the 2,3-diketo sugar followed by hydrolytic fission of the C-2 — C-3 and/or C-3 — C-4 bonds may be the way in which carbon-carbon bond cleavage is brought about in glucose oxidations:





Due to the high degree of oxidation of mesoxaldehyde, it may be unstable under reaction conditions and may break down to glyoxal and formic acid as suggested previously.

This mechanism accounts for the observed reaction products and stoichiometry. And based upon the present concept of the way in which ferric ion oxidations take place, it seems to be the most reasonable mechanism for carbon-carbon bond cleavage in glucose oxidations. However, the experimental evidence is insufficient for definitely establishing which cleavage mechanism is operative in glucose oxidations.

OXIDATION OF 3-O-METHYLGLUCOSE

The kinetics and products of ferric ion oxidations of glucose in 0.01M $HClO_4$ indicate that an initial product is rather easily oxidized, leading to splitting of a carbon-carbon bond. It has been proposed that the initial product of oxidation of glucose is glucosone and that secondary oxidation of the latter compound occurs at the C-3 hydroxyl, followed by hydrolytic cleavage of the C-3 — C-4 bond.

To test this hypothesis, the kinetics and products of 3-O-methylglucose oxidation were studied. It was reasoned that the initial oxidation product ought to be the osone as in glucose oxidations, but that secondary oxidation would not occur if the methoxyl group blocks oxidation of the osone at C-3. Thus, there ought to be no induction period, and no fission products in oxidations of 3-O-methylglucose if the mechanism which has been proposed for glucose oxidations is correct.

KINETICS

The results of a number of oxidations of 3-O-methylglucose by ferric perchlorate (10^{-3}) in 0.01M $HClO_4$ at 90.0°C. and at various substrate concentrations are shown in Fig. 22. The linear pseudo-first-order plots do not appear to have induction periods with increasing slopes as in oxidations of glucose. This, plus the fact that no evidence was obtained for the presence of 2- or 3-carbon carbonyl compounds in 3-O-methylglucose reaction mixture (as will be shown in the following section), supports the mechanism which has been proposed for the oxidation of glucose by ferric ion.

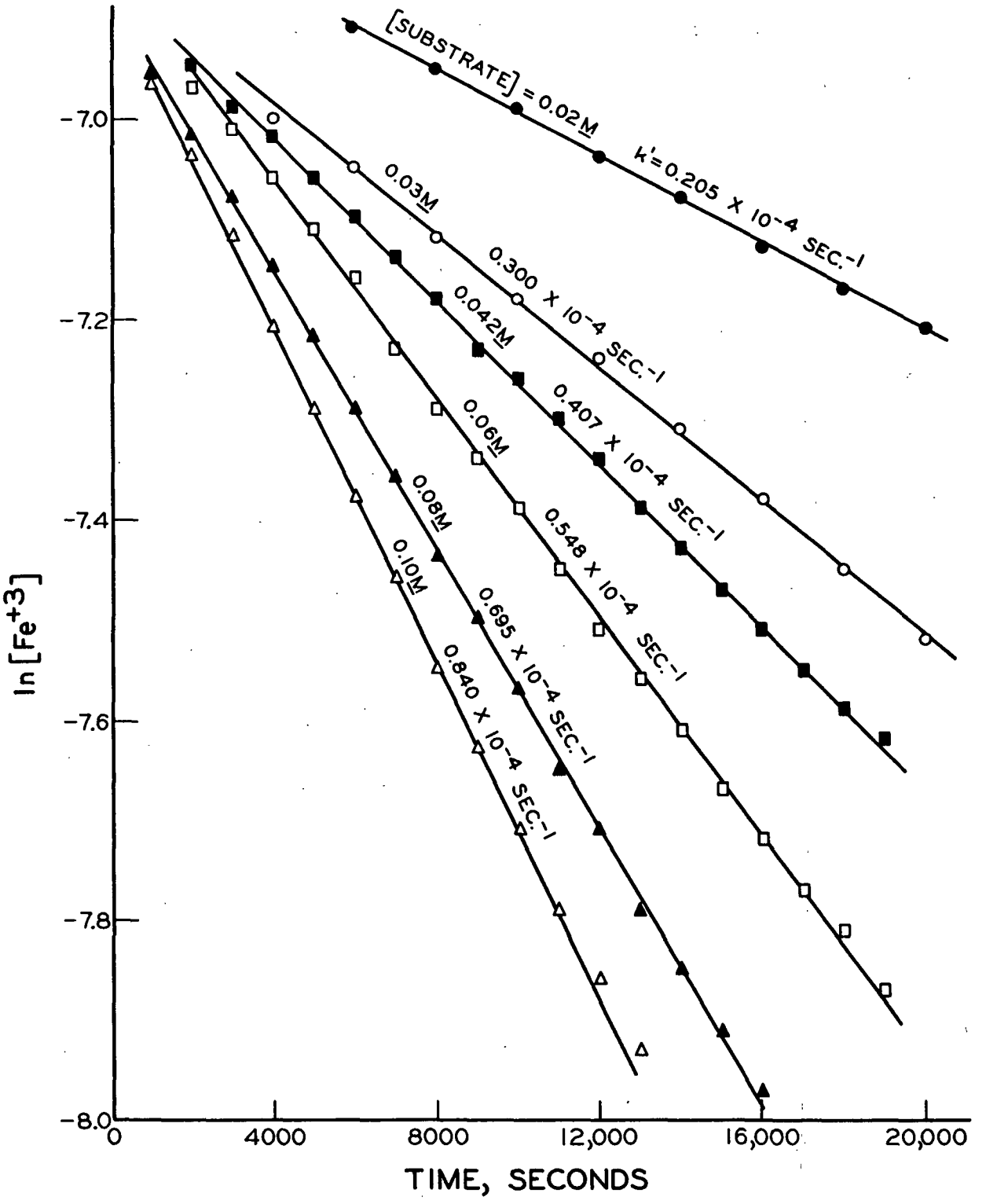


Figure 22. The Effect of Substrate Concentration on the Oxidation of 3-O-Methylglucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 90.0°C.

The proposed mechanism is given further support by a comparison of the rate constants of glucose and 3-O-methylglucose oxidations. The initial rate constant for the oxidation of glucose is thought to be that for the oxidation of glucose to glucosone, and is predicted to be equal to one-half the limiting rate constant which is eventually attained. If no secondary oxidation occurs in the case of 3-O-methylglucose, and if the initial step in oxidations of both glucose and 3-O-methylglucose is formation of the osone, the initial rate constant for the oxidation of glucose ought to be approximately equal to the rate constant for the oxidation of 3-O-methylglucose (assuming that replacement of glucose's C-3 hydroxyl group with a methoxyl group does not affect the rate of oxidation for the C-2 hydroxyl significantly). Comparison of Fig. 16 and 22 shows this to be true. For example, the limiting rate constant for the oxidation of 0.10M glucose is 1.70×10^{-4} sec.$^{-1}$, according to Fig. 16. From this, the initial rate constant is calculated to be 8.5×10^{-5} sec.$^{-1}$, which compares very favorably with the overall rate constant for the oxidation of 0.10M 3-O methylglucose, 8.4×10^{-5} sec.$^{-1}$.

When the pseudo-first-order rate constants for 3-O-methylglucose oxidations are plotted against substrate concentration (Fig. 23), a straight line is not obtained, as would be expected if the dependence upon substrate concentration were first-order. However, Duke's reciprocal plot (Fig. 24) gives an excellent straight line, good evidence for oxidation via complex formation. (Refer to p. 45.) Thus, the kinetics of 3-O-methylglucose oxidations are entirely in accord with the mechanism which has been proposed for glucose oxidations.

PRODUCT ANALYSIS

An oxidation of 3-O-methylglucose (0.02M) was carried out in 0.01M perchloric acid at 90.0°C. for 10.5 hours. The ferric ion concentration decreased from

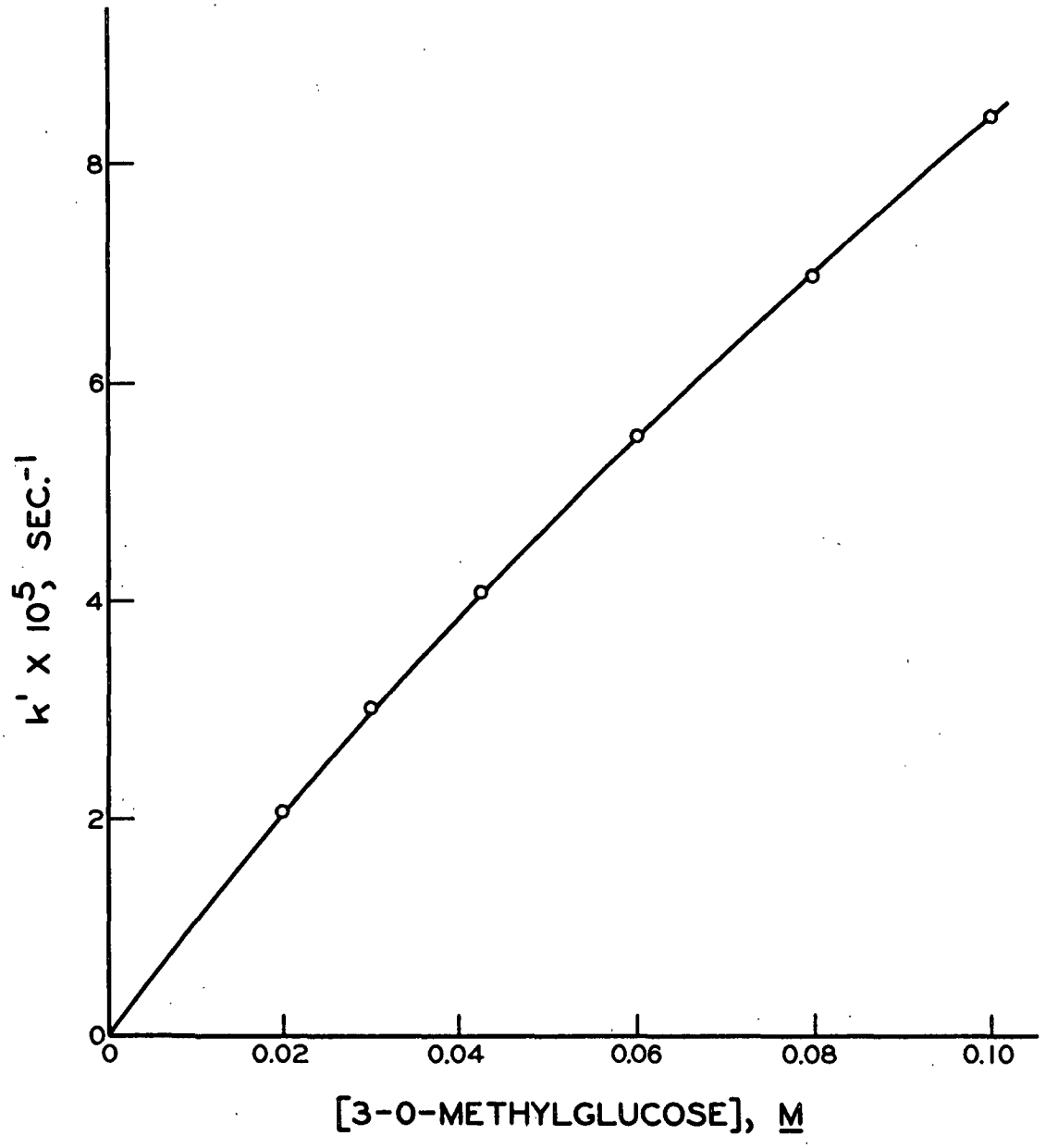


Figure 23. The Relationship Between Substrate Concentration and Reaction Rate Constant for the Oxidation of 3-O-Methylglucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 90.0°C.

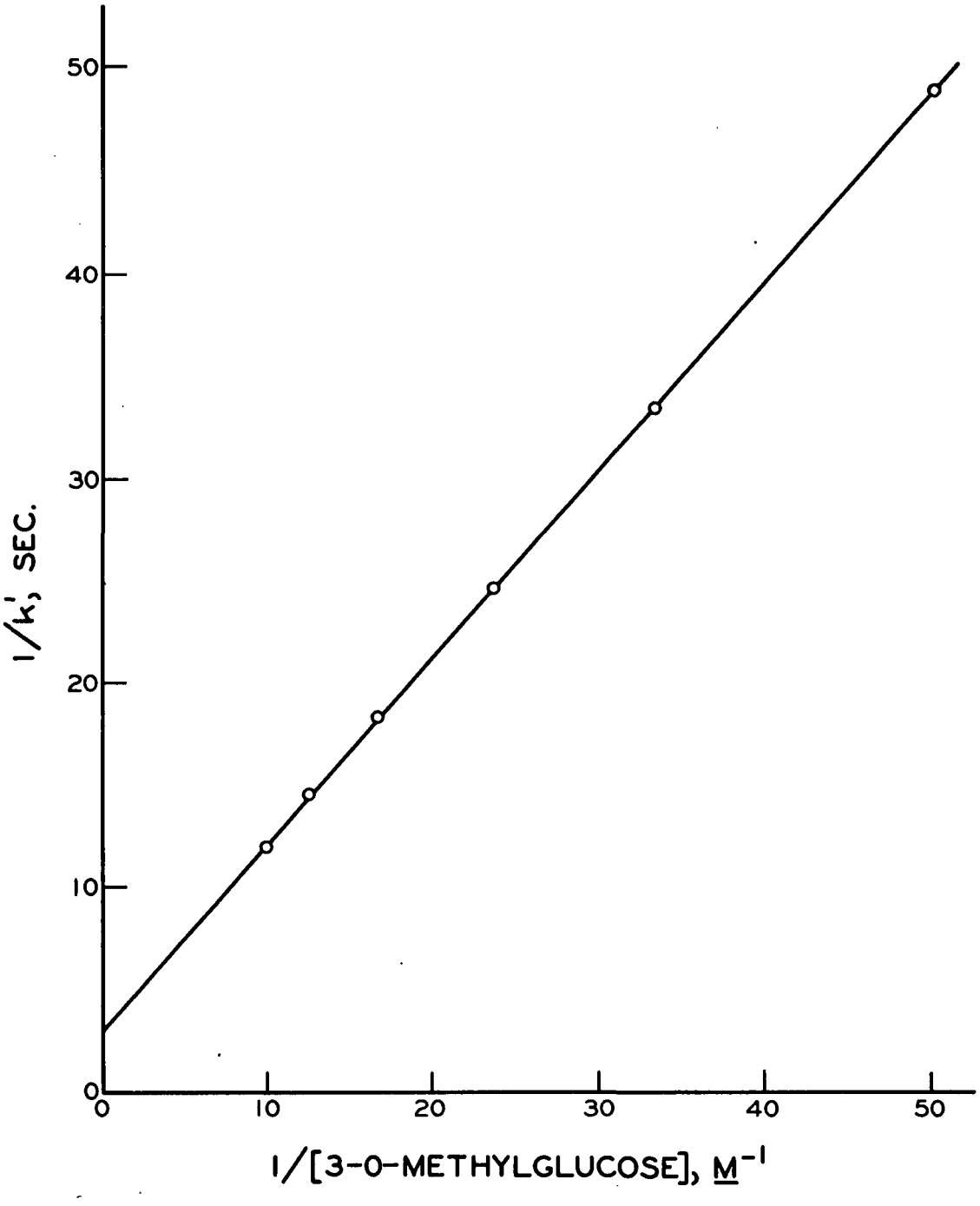


Figure 24. Duke's Reciprocal Plot for the Oxidation of 3-O-Methylglucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 90.0°C.

10.40×10^{-4} M to 2.32×10^{-4} M. A 50.0-ml. portion of the reaction mixture was treated with an equal volume of saturated 2,4-dinitrophenylhydrazine in 2M HCl at room temperature for 6 hours. The precipitate which was obtained weighed 5.7 mg., as compared to a 0.1-mg. precipitate from 50 ml. of unoxidized 0.02M 3-O-methylglucose solution.

Assuming that the product is the osone, and that two moles of ferric ion are consumed per mole of osone formed, the theoretical yield is calculated to be 11.2 mg. The observed yield is therefore 5.7 - 0.1/11.2 x 100% = 50% of theoretical.

The precipitate was chromatographed in benzene-tetrahydrofuran (80:20). Only one spot resulted and this had the same chromatographic mobility as the major component in a mixture of DNPH's obtained by heating 3-O-methylglucose with 2,4-dinitrophenylhydrazine in acid solution. This indicates that the product from oxidation of 3-O-methylglucose by ferric perchlorate is the corresponding osone. It is to be particularly noted that two- and three-carbon fragments were not detected by 2,4-dinitrophenylhydrazine.

Qualitatively, the product analysis results for 3-O-methylglucose support the conclusions derived from the kinetic study. The failure to obtain a quantitative yield of the 2,4-dinitrophenylhydrazine derivative has no apparent explanation. The fact that the yield came out to be exactly 50% of theoretical suggests that four rather than two moles of ferric ion may be required to produce the oxidation product. However, it is difficult to conceive of a mechanism having such a stoichiometry.

THE OXIDATION OF 2-DEOXYGLUCOSE

The oxidation of 2-deoxyglucose was studied in order to test the hypothesis that the C-2 hydroxyl of glucose is involved in the oxidation of the latter sugar by ferric ion. It was reasoned that if this hypothesis were true, 2-deoxyglucose ought to be relatively unreactive toward ferric ion since it has no C-2 hydroxyl.

But, as can be seen from Fig. 25, 2-deoxyglucose reduced ferric ion faster than did glucose under the same conditions. This apparently would indicate that a C-2 hydroxyl is not necessary in order for an aldose to be able to reduce ferric ion at an appreciable rate. However, it was learned that 2-deoxyglucose is much more susceptible to acid degradation than is glucose (62). Therefore, the possibility was considered that a degradation product rather than 2-deoxyglucose itself was responsible for the reduction of ferric ion.

The kinetics of oxidation of 2-deoxyglucose support this hypothesis. The pseudo-zero-order kinetics (see Fig. 25) are consistent with a mechanism involving degradation of 2-deoxyglucose in the rate-controlling step. The effect of increasing the acid concentration from 0.01M to 0.05M (Fig. 26) is to increase the reaction rate, whereas the usual behavior of ferric ion oxidations is the opposite of this. This result also supports the degradation hypothesis since degradation is acid-catalyzed.

Another point in support of the degradation hypothesis is that oxidation mixtures were yellow-colored at the conclusions of the reactions. In fact, the reaction mixture which was 0.05M in $HClO_4$ was quite yellow after the usual one hour temperature equilibration period in the constant-temperature bath (before addition of ferric perchlorate). Acid degradation of glucose is known to be accompanied by development of a yellow coloration, presumably due to condensation of 5-hydroxymethylfurfural (49).

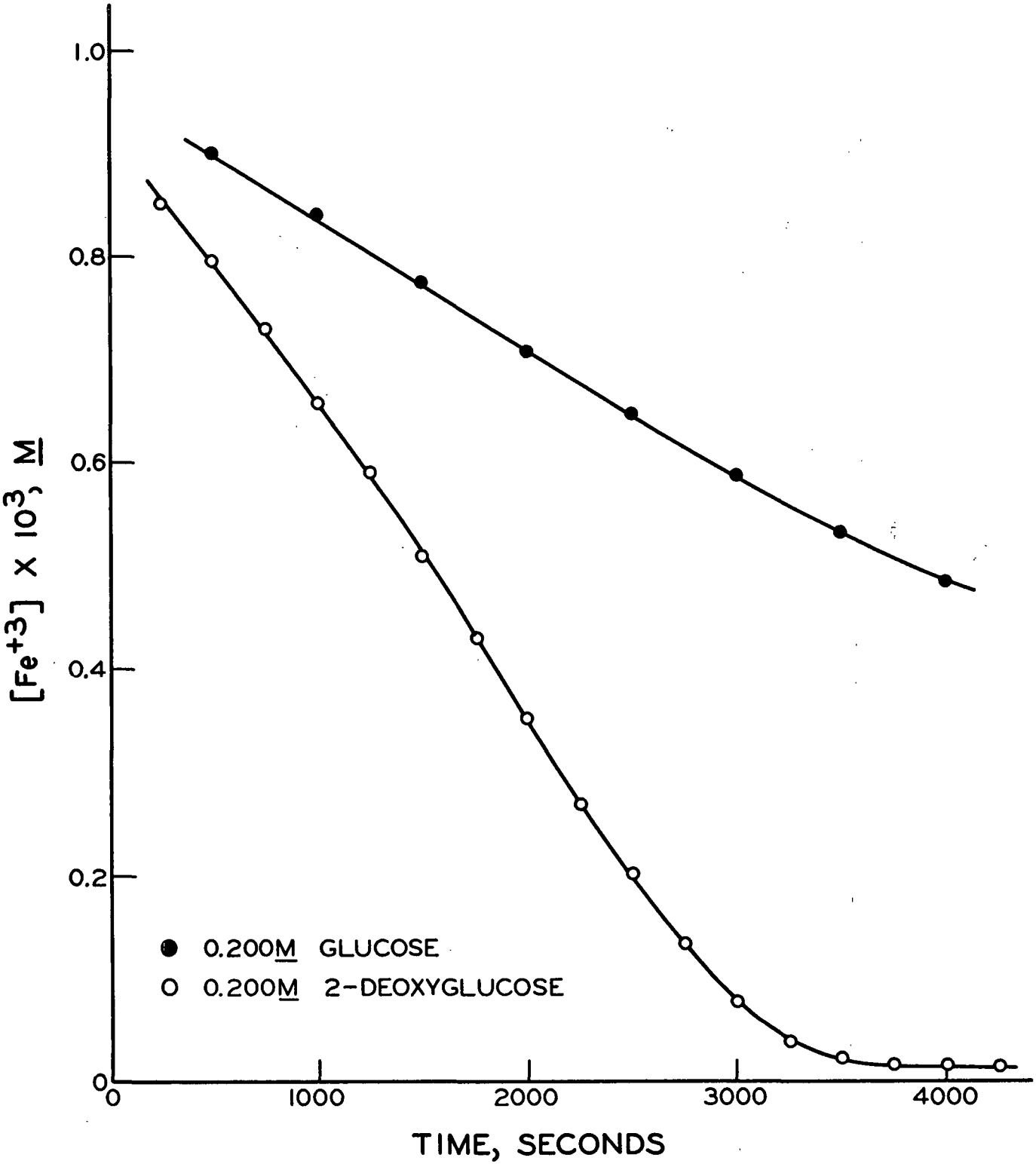


Figure 25. Oxidations of Glucose and 2-Deoxyglucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 88.6°C.

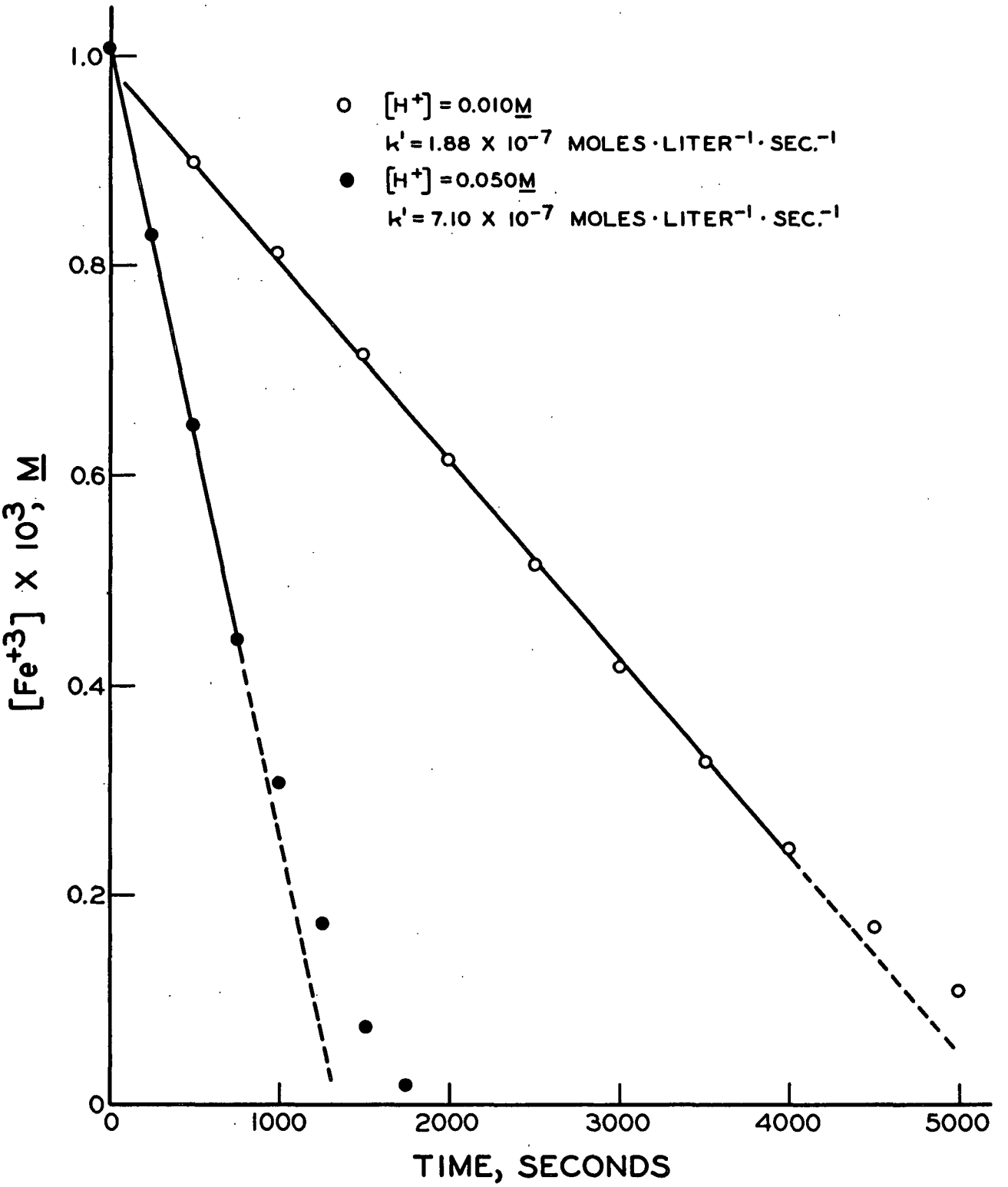


Figure 26. The Effect of Acid Concentration on the Oxidation of 0.0500M 2-Deoxyglucose by 10^{-3}M $Fe(ClO_4)_3$ at 90.0°C.

Thus, the apparent ability of 2-deoxyglucose to reduce ferric ion does not disprove the involvement of the C-2 hydroxyl of glucose in the ferric ion of the latter sugar. This is because it is probably not the 2-deoxyglucose itself which reduces ferric ion, but an acid degradation product of the deoxy sugar.

THE OXIDATION OF TETRA-O-METHYLGLUCOSE

The oxidation of 2,3,4,6-tetra-O-methylglucose was studied since it was thought that a methoxyl group at the 2-position would probably block complex formation with and oxidation by ferric ion, thereby providing evidence for the involvement of the C-2 hydroxyl in the ferric ion oxidation of glucose. The methoxyl groups at the remaining positions had no purpose, tetra-O-methylglucose being more readily available than 2-O-methylglucose.

But, as illustrated by Fig. 27, the oxidation of 2,3,4,6-tetra-O-methylglucose was actually faster than the oxidation of glucose. This is similar to the situation encountered by Pottenger (20) in which the oxidation of 2-O-methylglucose by ceric ion was faster than the oxidation of glucose. It may be that the methoxyl group at C-2 does not block oxidation at this position, since the methoxyl oxygen has unshared electron pairs which might engage in a coordination complex with the metal ion. On the other hand, the methoxyl group of 3-O-methylglucose appears to block oxidation at the 3-position (p. 70).

The reaction curve shown for tetra-O-methylglucose in Fig. 27 has two peculiar features. First, although the initial ferric ion concentration was known to be 1.0×10^{-3} M, extrapolation of the reaction curve to zero time indicates an initial concentration of only about 0.75×10^{-3} M. And secondly, the reaction curve levels out at a ferric ion concentration of about 0.1×10^{-3} M, indicating completion of the reaction before all of the ferric ion was consumed.

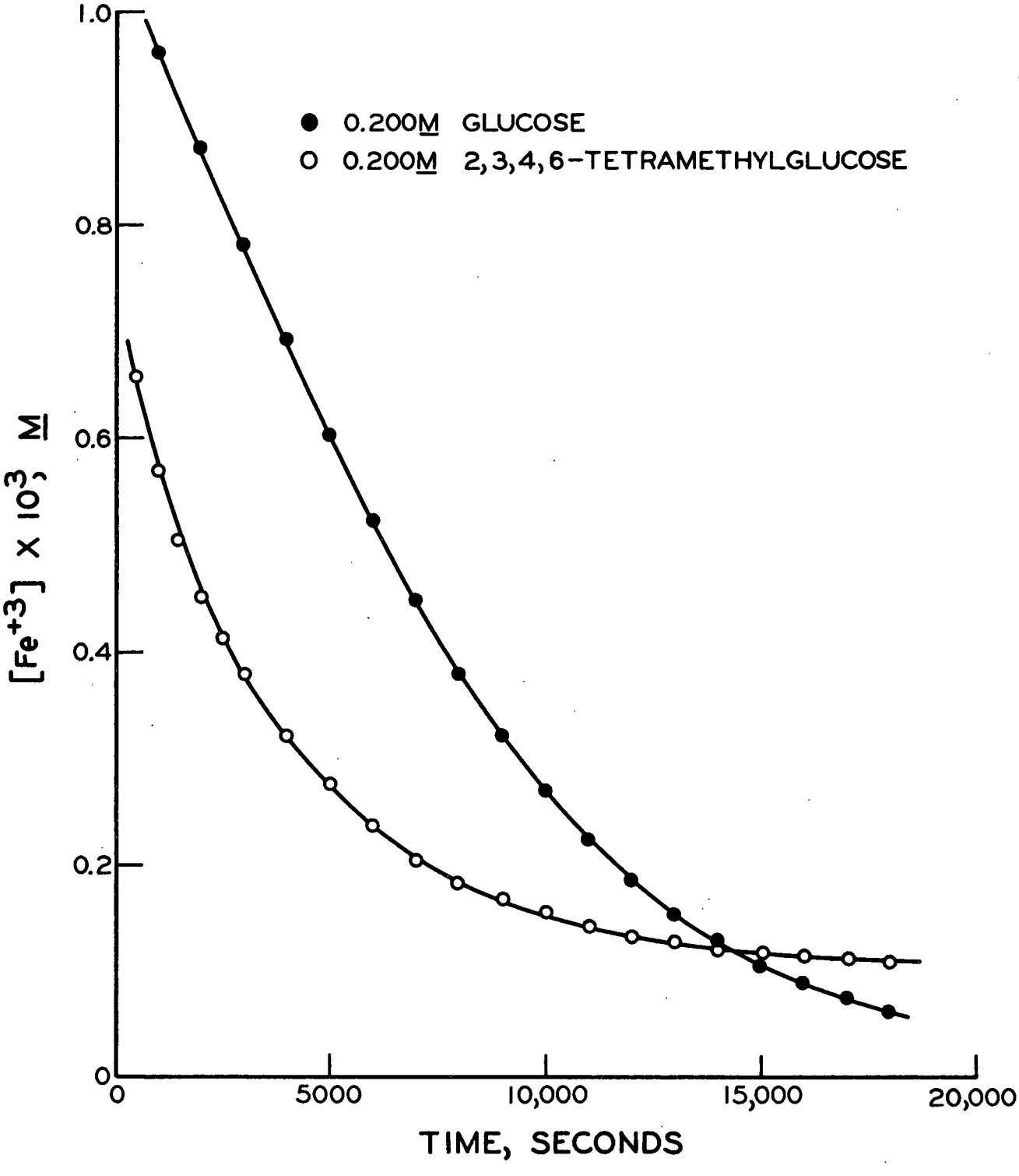


Figure 27. Oxidations of 2,3,4,6-Tetra-O-methylglucose and Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 88.6°C.

These features were duplicated in a second run carried out under the same conditions. The reason for these features and for the unexpected reactivity of tetra-O-methylglucose are not known.

MODEL COMPOUND STUDY

From the results of studies on glucose and 3-O-methylglucose oxidations, a mechanism has been proposed for the ferric ion oxidation of these sugars. It appears that their reactivity is the result of a highly specific reaction between ferric ion and the C-2 alcohol group. Other alcohol groups, the ether linkage, and the potential carbonyl function do not seem to be attacked by ferric ion. The special reactivity of the C-2 alcohol group is thought to be a consequence of its adjacency to a carbonyl group. The α-hydroxycarbonyl grouping is hypothesized to be highly reactive toward ferric ion.

The model compound study tests the conclusions which have been reached concerning the reactivities of the various groupings in glucose. Compounds containing one or more functional groupings were examined for reactivity toward ferric ion. Kinetic and product studies of compounds which were found to be reactive were carried out to determine reaction mechanisms. These reaction mechanisms were then compared with the mechanism proposed for glucose oxidations.

MONOHYDRIC ALCOHOLS

Secondary Alcohols

Cyclohexanol was chosen to test the reactivity of the secondary alcohol group. Figure 28 shows the results of an oxidation of cyclohexanol (0.20M) by ferric perchlorate (0.0010M) in 0.01M $HClO_4$ at 88.6°C. An oxidation of glucose under similar conditions is shown for comparison.

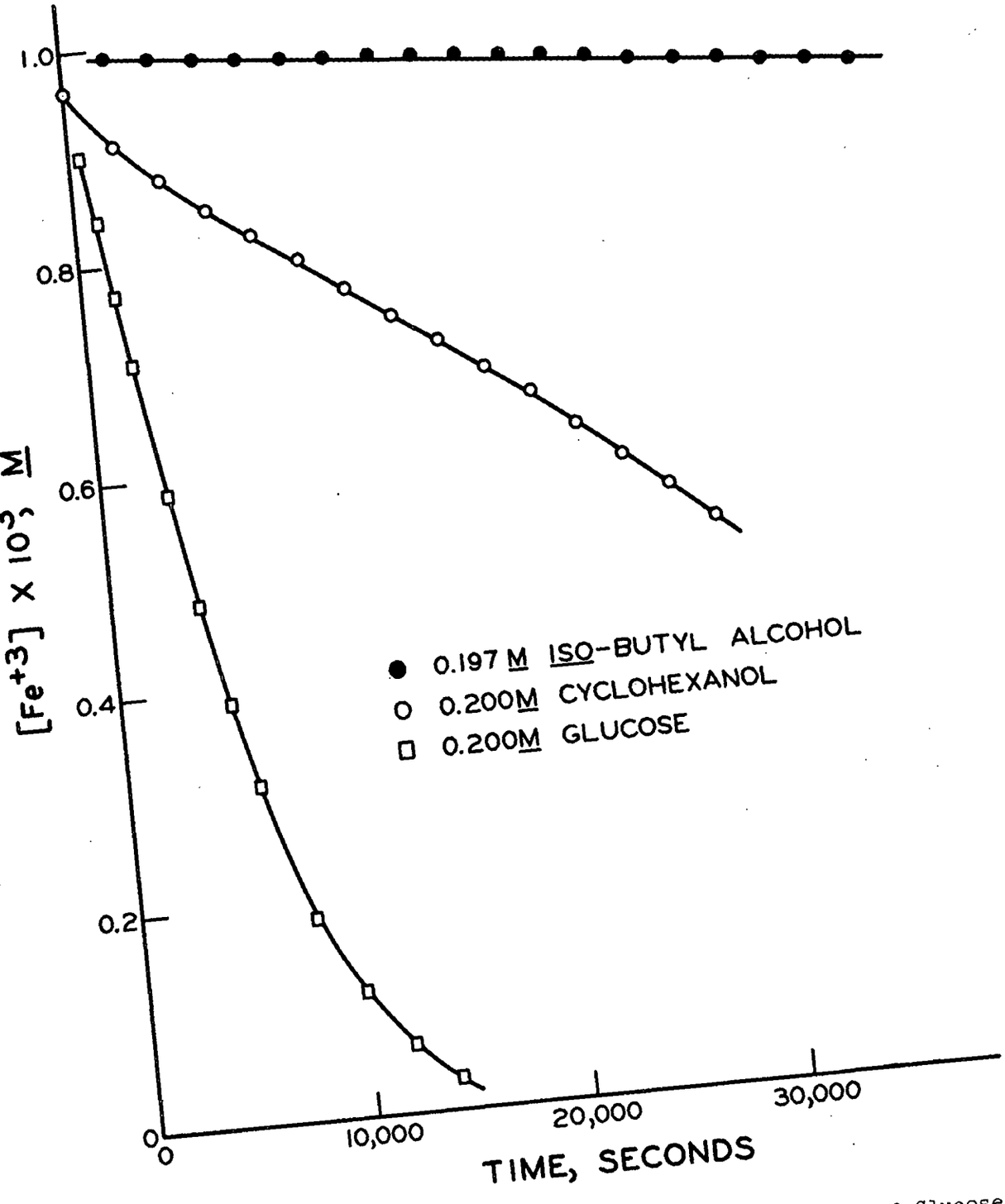


Figure 28. Oxidations of Cyclohexanol, Isobutyl Alcohol, and Glucose in 0.0100M $HClO_4$ at 88.6°C. by 10^{-3}M $Fe(ClO_4)_3$.

The data from the oxidation of cyclohexanol shown in Fig. 28 fit pseudo-zero-order and pseudo-first-order kinetics equally well. The pseudo-first-order rate constant is 2.15×10^{-5} sec.$^{-1}$, as compared to a value of 2.95×10^{-4} sec.$^{-1}$ for a similar oxidation of glucose. This indicates a surprisingly high reactivity for the secondary alcohol group, since glucose is oxidized only about 14 times faster than cyclohexanol.

However, there are a number of reasons for suspecting that most of cyclohexanol's apparently high reactivity is due to a trace amount of a highly reactive impurity. One reason is that different cuts from fractional distillation of cyclohexanol give significantly different reaction rates. Also, the reaction rate of a particular cyclohexanol sample increased after being stored a while. The latter suggests that the reactive impurity in cyclohexanol may be cyclohexanone, resulting from air oxidation of the alcohol. This was supported by the finding that 0.20M cyclohexanone is oxidized too rapidly in 0.01M $HClO_4$ by 10^{-3}M ferric perchlorate at 88.6°C. to be followed by the automatic sampling and analysis method.

Another reason for suspecting that the true reactivity of cyclohexanol is lower than its apparent reactivity is the fact that cyclohexanol appears to reduce ferric ion about 8 times faster than cis- or trans-1,2-cyclohexanediol. (Refer to p. 82.) Transition metal ion oxidants usually oxidize α-glycols more readily than monohydric alcohols.

The final reason for suspecting that cyclohexanol contains a highly reactive impurity is that another monohydric, secondary alcohol, 2-butanol, was found to

reduce ferric ion much more slowly than does cyclohexanol. (See Fig. 29.*) Therefore, the true reactivity of the secondary alcohol group is probably fairly low in comparison with glucose.

Primary Alcohol

Also shown in Fig. 28 is the oxidation of iso-butyl alcohol (0.20M) in 0.01M $HClO_4$ at 88.6°C. The pseudo-first-order rate constant for the oxidation of this compound is 2.35×10^{-6}, 125 times less than that for glucose, showing that the primary alcohol group is not especially reactive toward ferric ion.

GLYCOLS

trans-1,2-Cyclohexanediol

Oxidations of 0.20M trans-1,2-cyclohexanediol at 88.6°C. are shown in Fig. 30 at three acid concentrations. The pseudo-first-order rate constant for the oxidation of trans-1,2-cyclohexanediol in 0.01M $HClO_4$ is 2.75×10^{-6} sec.$^{-1}$. Glucose, whose pseudo-first-order rate constant under the same conditions is 2.95×10^{-4} sec.$^{-1}$, is oxidized over 100 times faster than trans-1,2-cyclohexanediol. Therefore, the comparatively high reactivity of glucose toward ferric ion cannot be attributed to its trans-α-glycol groupings.

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*Oxidation of 2-butanol by ferric perchlorate could not be carried out in 0.01M $HClO_4$. At this acidity, an iron compound precipitated from solution when 2-butanol was the substrate (verified by duplication). This is apparently the result of a very specific interaction between ferric ion and 2-butanol, since precipitation never occurred under the same conditions with any of the other substrates. Oxidations of cyclohexanol and 2-butanol were compared in 0.03M $HClO_4$, in which ferric ion — 2-butanol solutions were found to be stable.

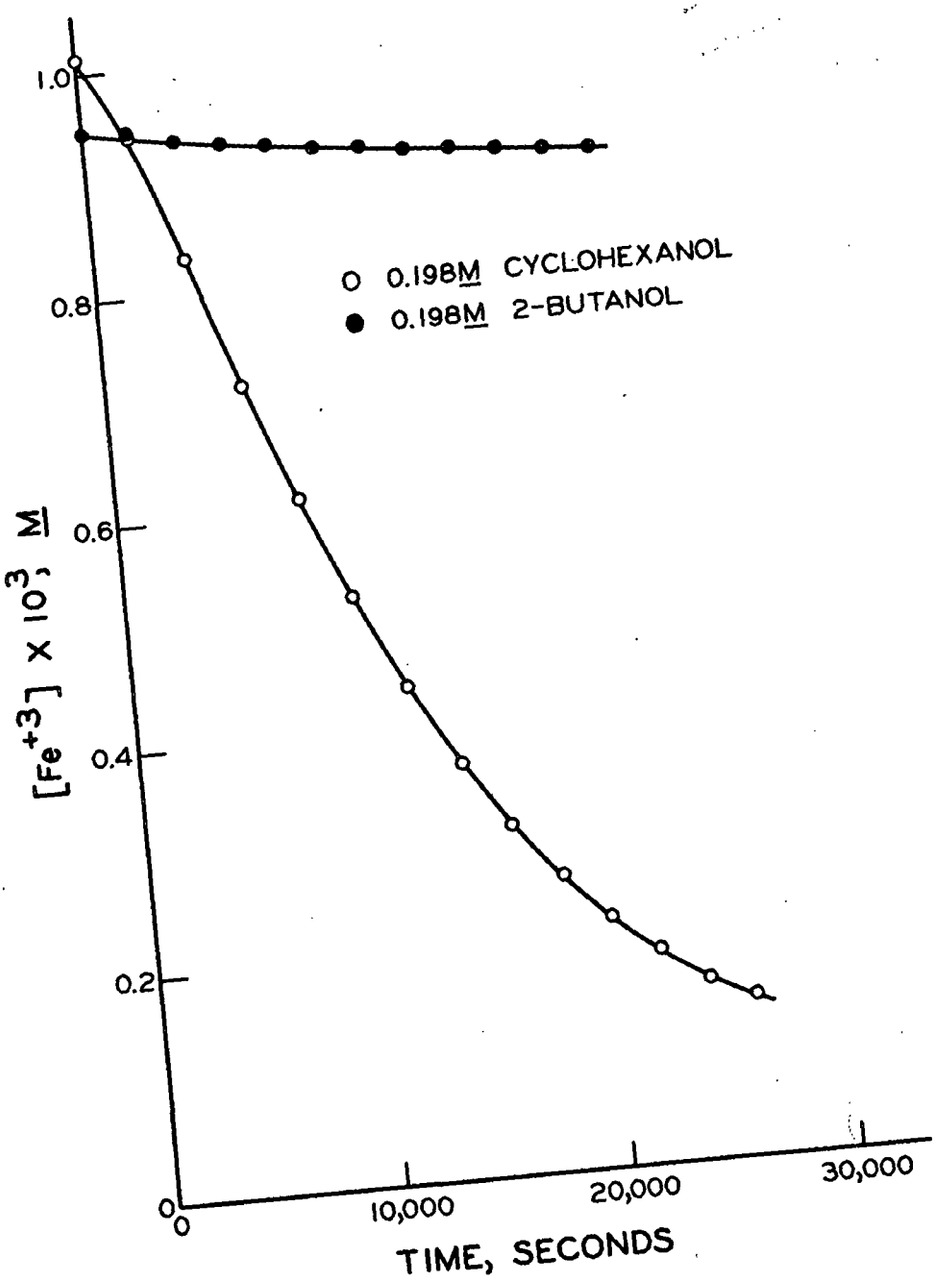


Figure 29. Oxidations of Cyclohexanol and 2-Butanol by 10^{-3}M $Fe(ClO_4)_3$ in 0.0300M $HClO_4$ at 88.6°C.

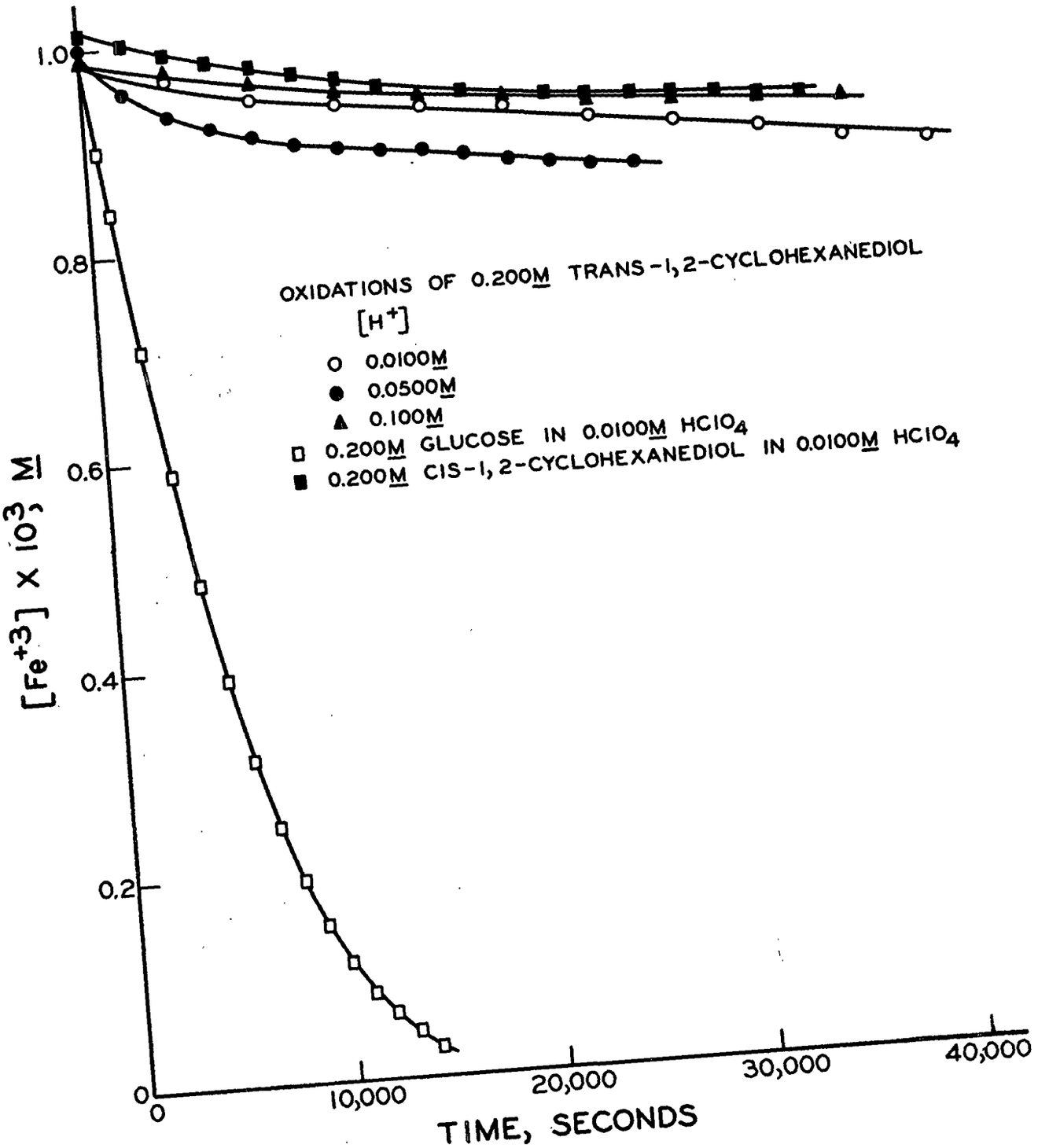


Figure 30. Oxidations of trans-1,2-Cyclohexanediol, cis-1,2-Cyclohexanediol, and Glucose by 10^{-3}M $Fe(ClO_4)_3$ at 88.6°C.

cis-1,2-Cyclohexanediol

Some oxidants show a preference for cis-α-glycols over trans-α-glycols. Since the α-anomer of glucose contains a cis-α-glycol grouping at C-1 — C-2, the reactivity of this grouping toward ferric ion was tested with cis-1,2-cyclohexanediol. The results in Fig. 30 show that the cis-diol is oxidized at about the same rate as the trans-diol in 0.01M $HClO_4$, indicating that a cis-orientation of adjacent hydroxyl groups is no more reactive toward ferric ion than a trans-orientation.

1,5-Anhydroglucitol

The reactivity of glucose is hypothesized to be a result of its ability to convert from a cyclic hemiacetal structure to an acyclic α-hydroxyaldehyde. If this is true, 1,5-anhydroglucitol, whose structure is similar to that of glucose except that it possesses no C-1 hydroxyl, hence no potential aldehyde function, should be relatively unreactive toward ferric ion in comparison with glucose.

That this is the case is shown by the results given in Fig. 31. The pseudo-first-order rate constant for the oxidation of 0.20M 1,5-anhydroglucitol at 88.6°C. in 0.01M $HClO_4$ is 8.40×10^{-6} sec.$^{-1}$, 35 times less than that of glucose. This establishes that the high degree of reactivity of glucose is due either to its C-1 hydroxyl group (in the cyclic form) or to the aldehyde function (the acyclic form). Since the product of oxidation of glucose does not appear to be gluconic acid (p. 62), it is improbable that the C-1 alcohol group in glucose is attacked by ferric ion, leaving the potential aldehyde function of glucose as the group which accounts for the high reactivity of the latter compound compared with 1,5-anhydroglucitol.

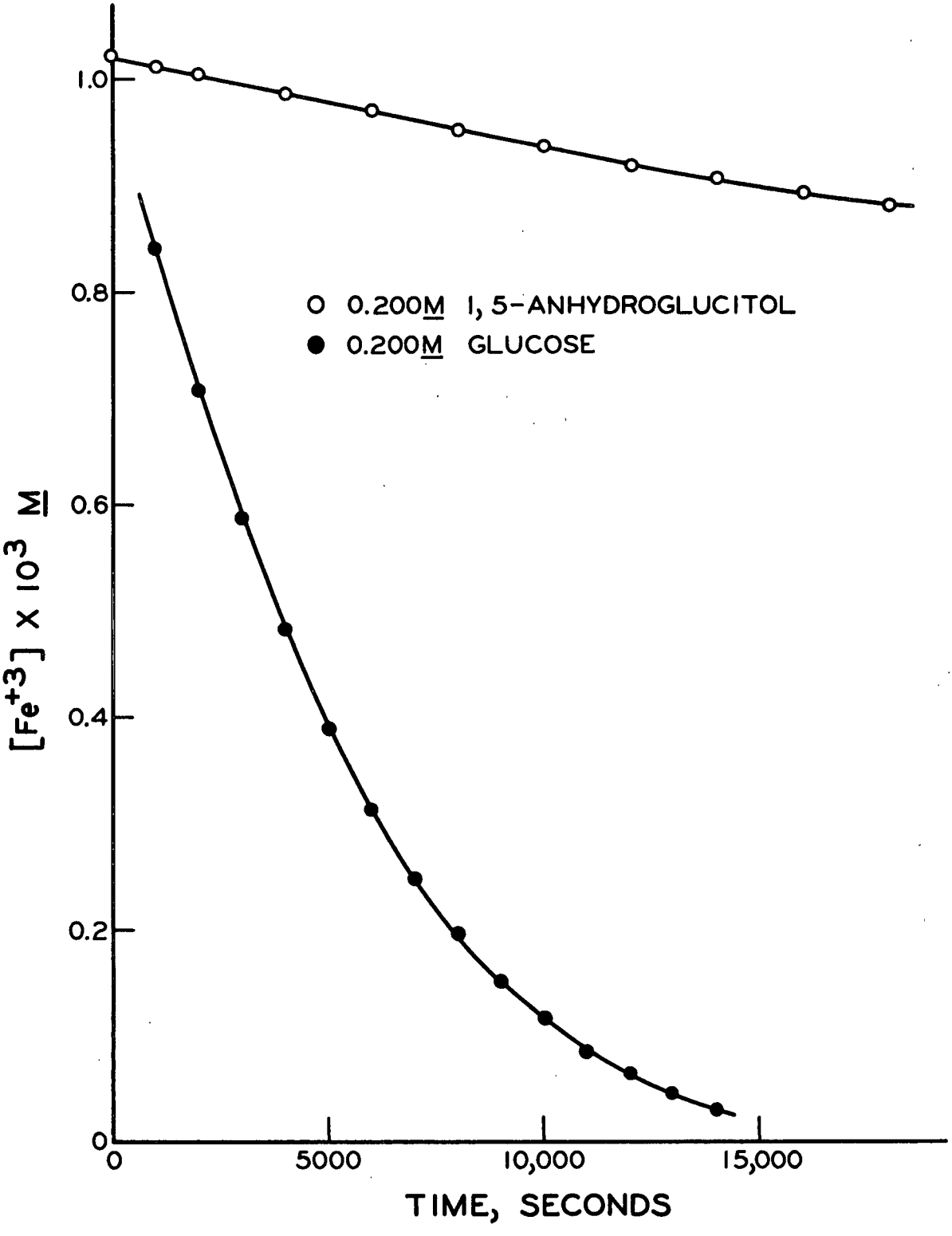


Figure 31. Oxidations of 1,5-Anhydroglucitol and Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 88.6°C.

BUTYRALDEHYDE

The reactivity of glucose is believed to be due to its α-hydroxyaldehyde grouping. It has been demonstrated that alcohol groups which are not adjacent to a carbonyl group reduce ferric ion slowly in comparison with glucose. Butyraldehyde was studied in order to determine whether an aldehyde function with no α-hydroxyl group is also relatively unreactive toward ferric ion.

Since it boils at 74°C. (63), butyraldehyde could not be oxidized at 88.6°C. for comparison with glucose. Therefore, it was compared against glycolaldehyde at 50.0°C. The data in Fig. 32 show that 0.20M butyraldehyde did not reduce ferric ion at all in 0.01M $HClO_4$, whereas 0.01M glycolaldehyde showed a significant reaction rate under the same conditions. This confirms the hypothesis that both the hydroxyl group and the carbonyl function of an α-hydroxycarbonyl grouping are involved in the reduction of ferric ion by the latter.

GLYCOLALDEHYDE

It has been shown that the aldehyde function and various arrangements of hydroxyl groups are relatively unreactive toward ferric ion. This is in accord with the hypothesis that the reactivity of glucose is due to the small fraction which is in the acyclic form, which contains an α-hydroxyaldehyde grouping. The oxidation of this grouping was studied in glycolaldehyde, the simplest α-hydroxyaldehyde, in order to determine the reaction mechanism.

Kinetics

The Effect of Acid Concentration

Glycolaldehyde (0.01M) was oxidized at 70.0°C. by 10^{-3} M ferric perchlorate at a number of acid concentrations (ionic strength maintained at 1.0 by addition

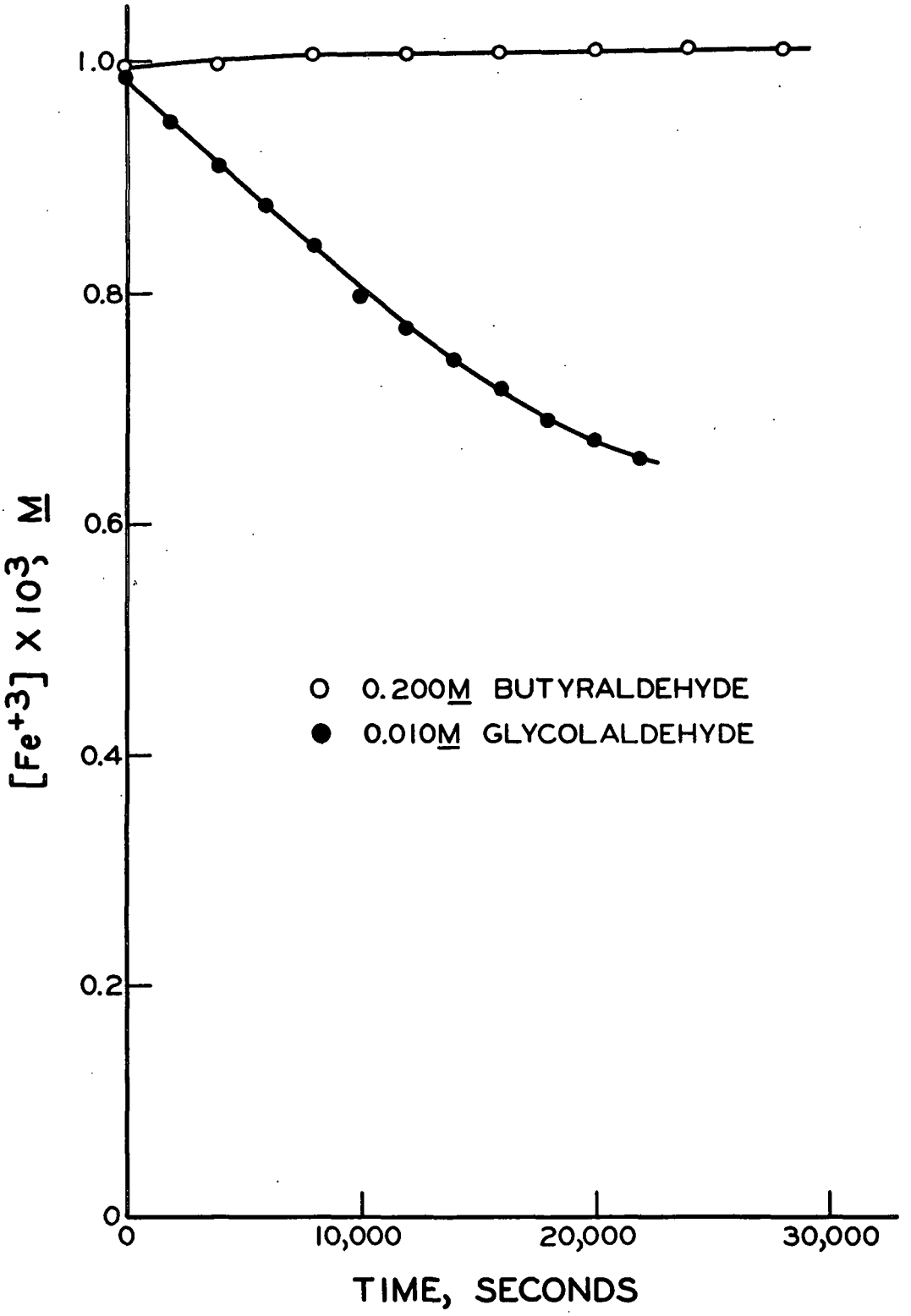


Figure 32. Oxidations of Butyraldehyde and Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in ~0.0100M $HClO_4$ at 50.0°C.

of sodium perchlorate). The effect of varying the acid concentration is shown in Fig. 33. As in the case of glucose, increasing the acid concentration decreases the reaction rate at low acidities, whereas the effect at higher acidities is the opposite of this. And, as in glucose oxidations, reaction kinetics are different at high and low acidities. But here the similarity ends, for oxidations of glycolaldehyde change from pseudo-first-order reactions to pseudo-zero-order reactions upon going from high to low acidities (Fig. 34 and 35), whereas the behavior of glucose is the reverse of this.

For glucose, the change in reaction order with a change in acid concentration was attributed to a change in the reaction mechanism (from oxidation of the 1,2-enediol to oxidation of a dehydration product). But, for glycolaldehyde, a mechanistic change is unlikely because the reaction product is glyoxal in both high and low acidity media (see p. 114). A more probable explanation of the change in reaction order is that changing the acid concentration changes the rate-controlling step of the reaction.

The effect of acid concentration upon reaction kinetics is consistent with the following reaction scheme,

$$\text{Aldehyde} \underset{}{\overset{\text{Step 1}}{\rightleftharpoons}} \text{Enediol} \xrightarrow[Fe^{+3}]{\text{Step 2}} \text{Products.}$$

The rate of oxidation of glycolaldehyde in 0.1M $HClO_4$ by 10^{-3}M ferric perchlorate is zero-order with respect to ferric ion concentration over most of the reaction, suggesting that Step 1 is rate-controlling under these conditions. Increasing the acid concentration would increase the rate of enolization (Step 1) since this reaction is acid-catalyzed (64). From the oxidation of acetoin by ferric perchlorate (15) it appears that increasing the acid concentration would decrease the rate of the oxidation step (Step 2). These two effects could result

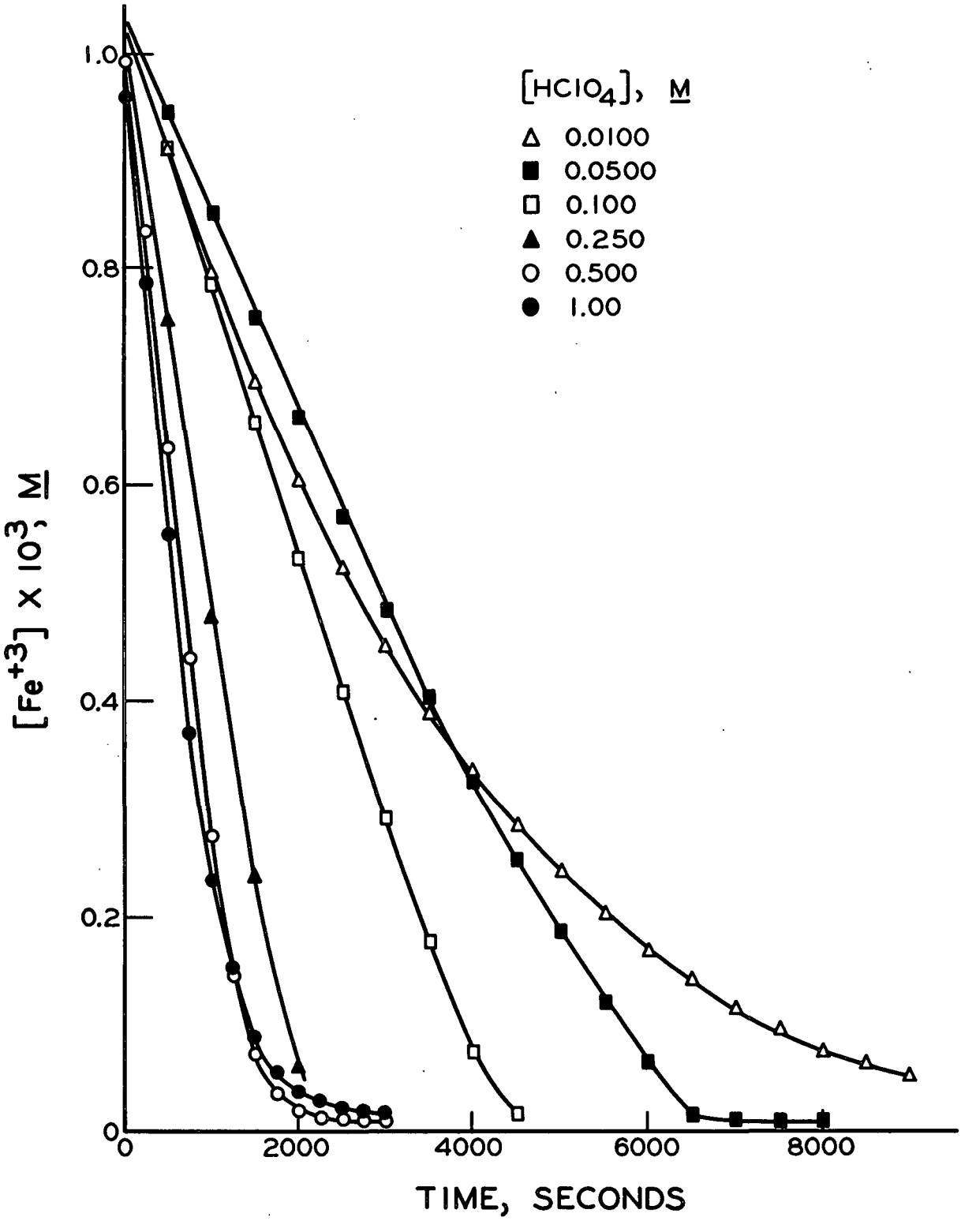


Figure 33. The Effect of Acid Concentration on the Rate of Oxidation of 0.0100M Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ at 70.0°C.

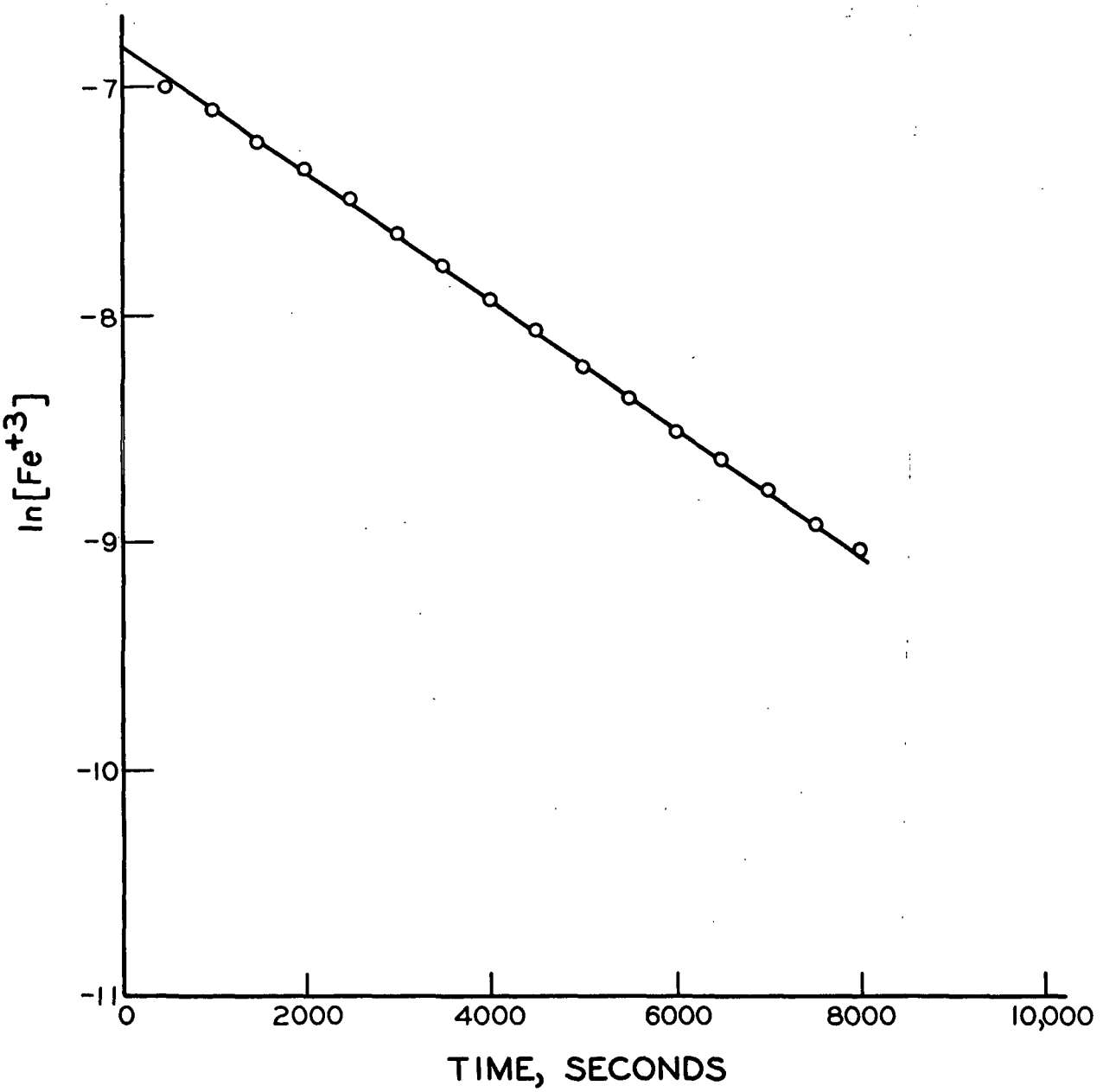


Figure 34. Pseudo-First-Order Plot for the Oxidation of 0.0250M Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 1.00M $HClO_4$ at 50.0°C.

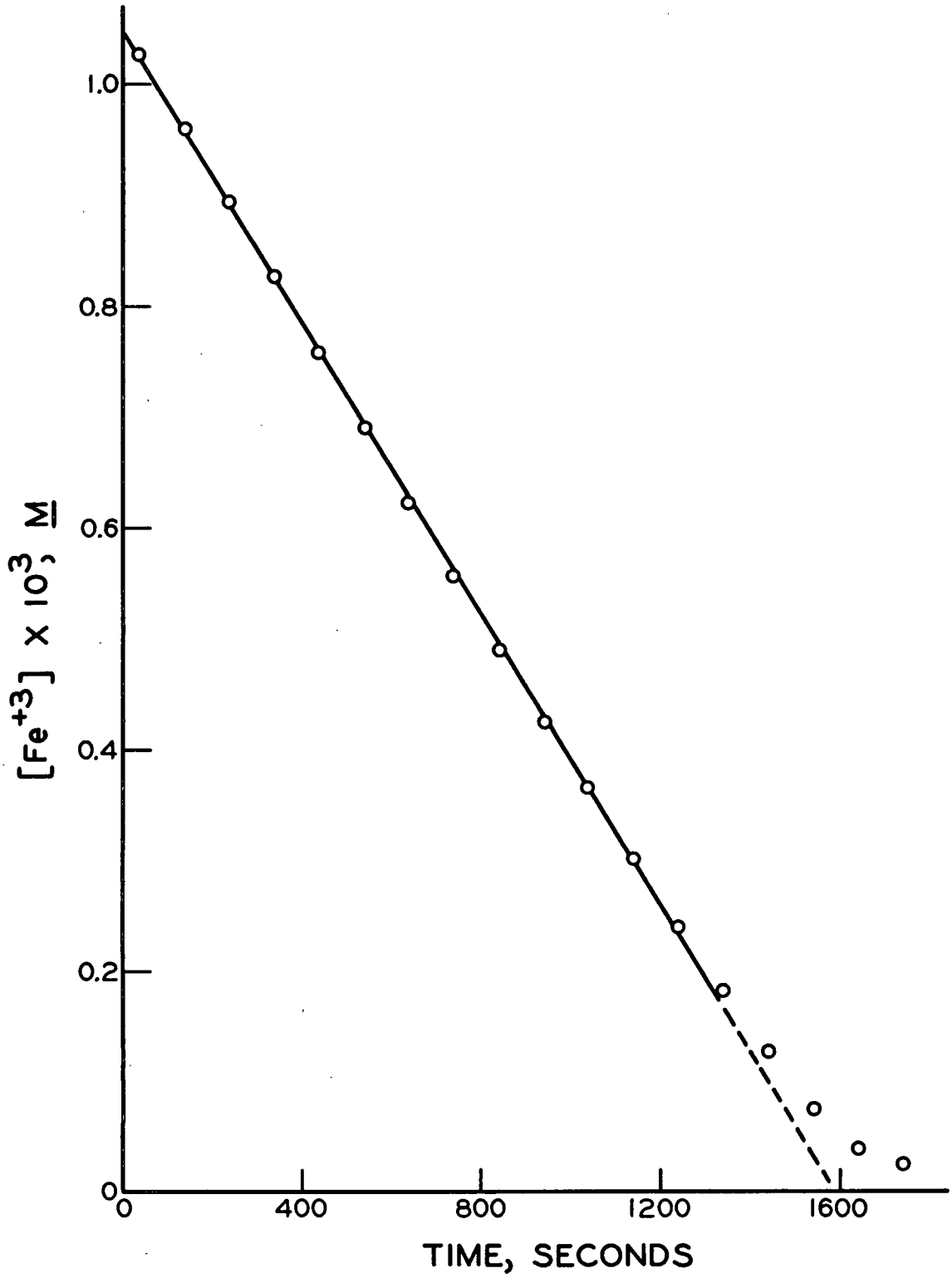


Figure 35. Pseudo-Zero-Order Plot for the Oxidation of 0.0250M Glycolaldehyde by 10^{-3} M $Fe(ClO_4)_3$ in 0.100M $HClO_4$ at 70.0°C.

in a change of the rate-controlling step from enolization at low acidities to oxidation at high acidities. This is consistent with the observed change of kinetics from pseudo-zero-order to pseudo-first-order upon going from 0.1M $HClO_4$ to 1M $HClO_4$.

The Effect of the Initial Ferric Ion Concentration

It was shown in the preceding section that the dependence upon ferric ion changes from zero-order to first-order upon increasing the acid concentration from 0.1M to 1M. It was proposed that this is a result of changing the relative rates of enolization and oxidation in a reaction mechanism having at least two steps.

It should be possible to bring about a similar transformation of kinetics by lowering the initial ferric ion concentration. In 0.25M $HClO_4$, pseudo-zero-order kinetics are followed over most of the course of a reaction at 60.0°C. between 0.01M glycolaldehyde and 1×10^{-3} M ferric perchlorate. (See Fig. 36.) This is interpreted as showing that enolization is rate-controlling at this acid concentration and this initial ferric ion concentration. Lowering the initial ferric ion concentration will decrease the rate of oxidation of the enediol without affecting the rate of the enolization reaction. If the initial ferric ion concentration is lowered sufficiently, the oxidation step will become rate-controlling and the overall reaction should exhibit pseudo-first-order kinetics. This is shown by Fig. 37 to be the case when the initial ferric ion concentration is 0.05×10^{-3} M. This supports the hypothesis which was proposed for the effect of acid concentration upon the oxidation of glycolaldehyde.

Rocek and Riehl (25) in a study of the chromic acid oxidation of ketones, found that the order of reaction with respect to chromic acid changed from first-order at low initial chromic acid concentrations to zero-order at high

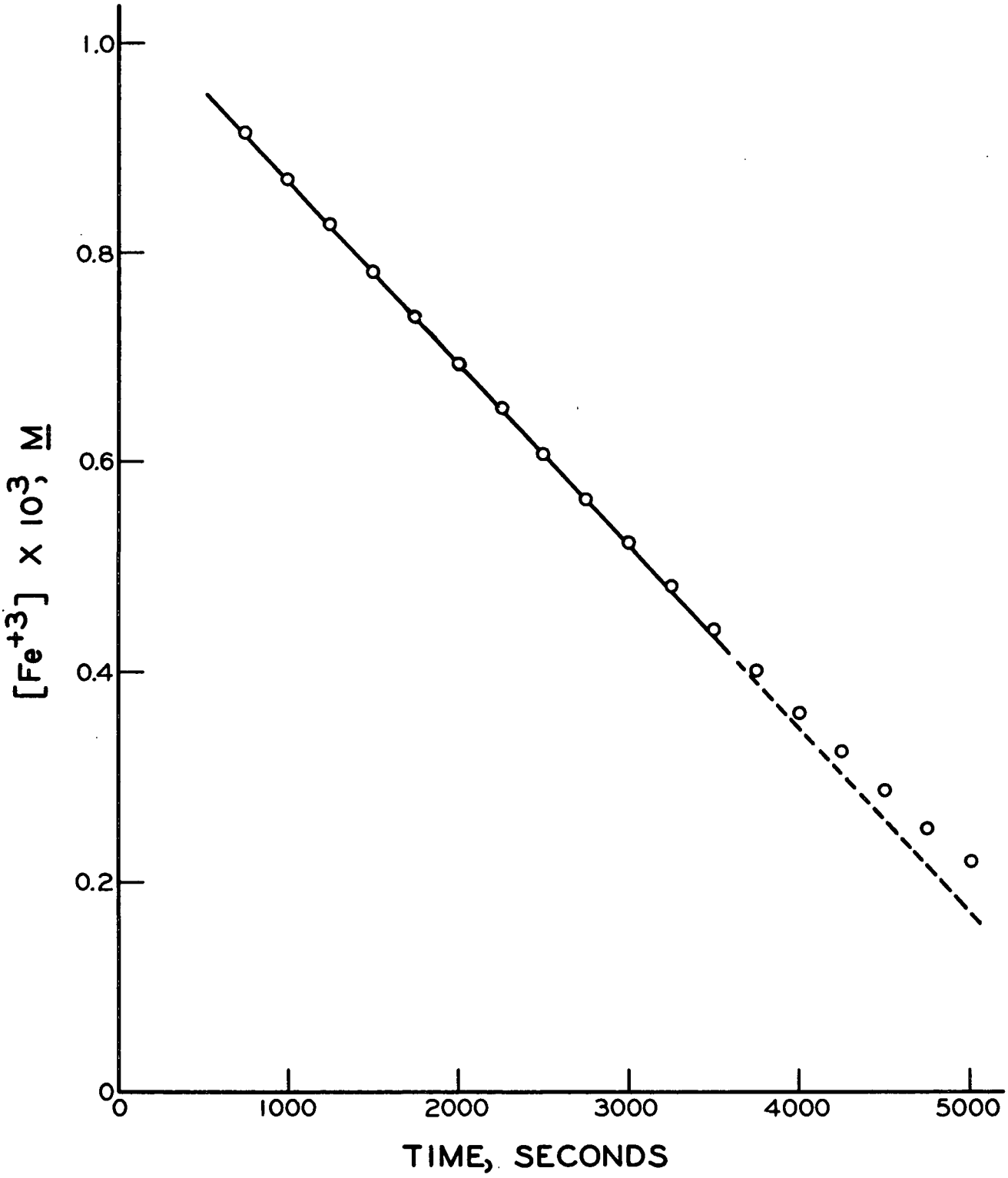


Figure 36. Pseudo-Zero-Order Plot for the Oxidation of 0.0100M Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.250M $HClO_4$ at 60.0°C.

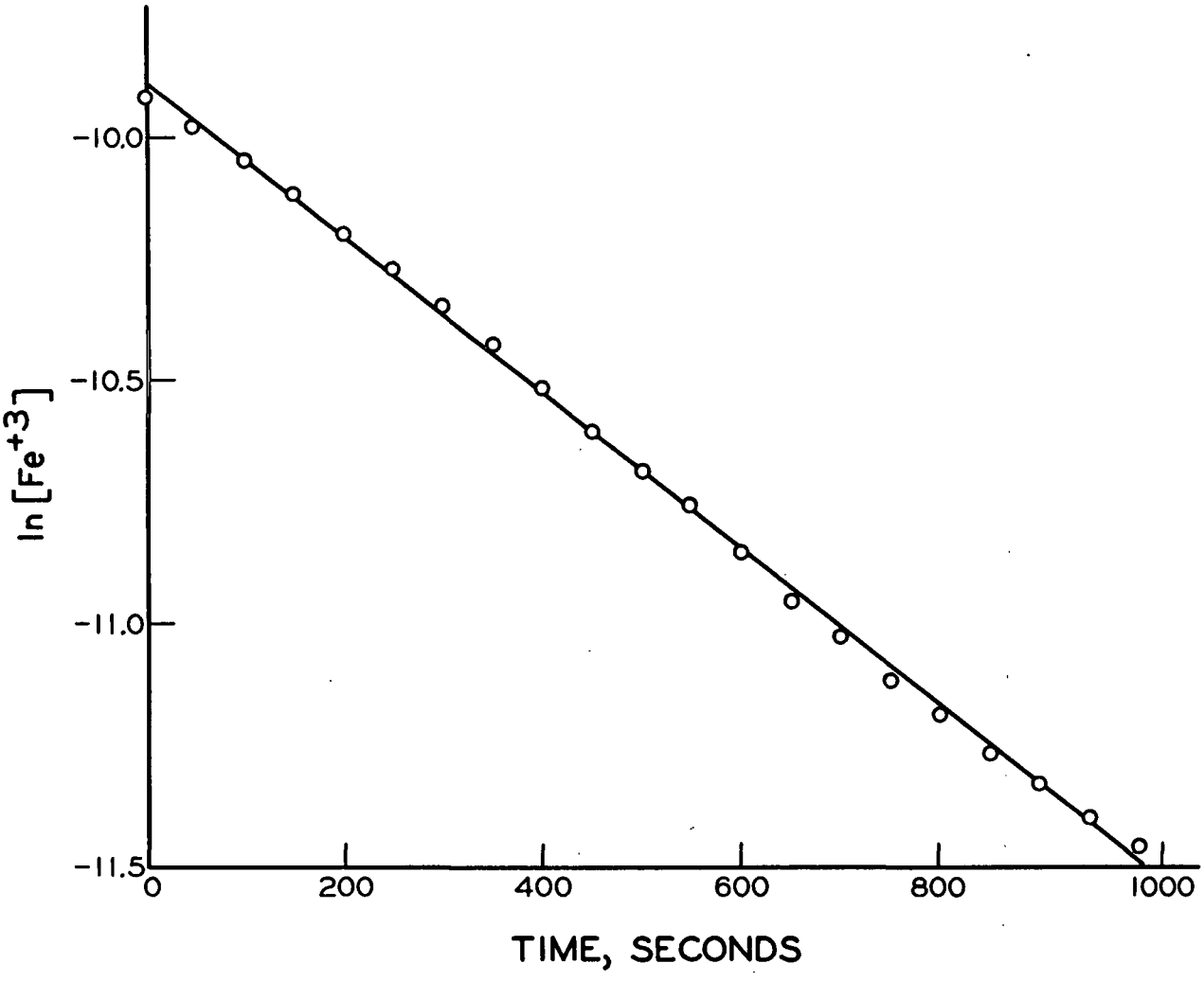


Figure 37. Pseudo-First-Order Plot for the Oxidation of 0.0100M Glycolaldehyde by 0.05 x 10^{-3}M $Fe(ClO_4)_3$ in 0.250M $HClO_4$ at 60.0°C.

initial chromic acid concentrations. They showed that, at high chromic acid concentrations, the rates of enolization of the ketones studied were equal to their rates of oxidation (taking into account the stoichiometries of the reactions involved). A kinetic expression, based on an enol oxidation mechanism, was derived for the range of chromic acid concentration in which rates of enolization and oxidation are comparable. The reasoning of Rocek and Riehl was as follows.

For the system,

$$\text{Ketone} \underset{k_K}{\overset{k_E}{\rightleftharpoons}} \text{Enol} \xrightarrow[k]{CrO_3} \text{Product},$$

the following rate expression can be derived (by making the steady-state assumption for the enol concentration):

$$\text{rate} = \frac{-d[CrO_3]}{dt} = \frac{k_E\, k\, [\text{ketone}]\, [CrO_3]}{k_K + k\, [CrO_3]} . \qquad (17)$$

This expression predicts that the reaction will be first-order in chromic acid concentration when $\underline{k}[CrO_3] \ll \underline{k_K}$ and zero-order when $\underline{k}[CrO_3] \gg \underline{k_K}$.

Overall first- and second-order constants may be defined by the equations,

$$-d[CrO_3]/dt = k_1\, [\text{ketone}], \text{ and} \qquad (18)$$

$$-d[CrO_3]/dt = k_2\, [\text{ketone}][CrO_3]. \qquad (19)$$

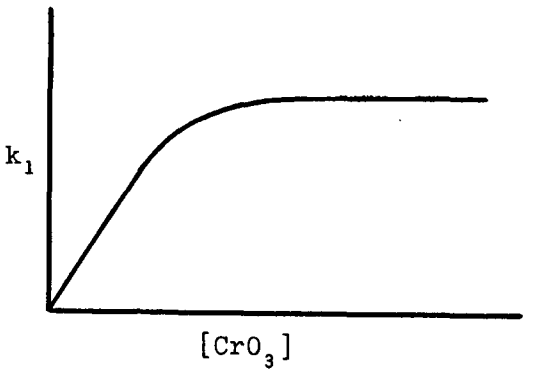
Solving for $\underline{k_1}$ and $\underline{k_2}$, one finds that

$$k_1 = k_E k[CrO_3]/(k_K + k[CrO_3]), \text{ and} \qquad (20)$$

$$k_2 = k_E k/(k_K + k[CrO_3]). \qquad (21)$$

At high $[CrO_3]$, Equation (20) simplifies to $k_1 = k_E$, and at low $[CrO_3]$, Equation (21) reduces to $k_2 = kk_E/k_K$.

If the initial chromic acid concentration is varied and values of k_1 are calculated from initial reaction rates, Equation (20) predicts that a plot of the form,



will be obtained. Oxidations of isobutyrophenone and 2-chlorocyclohexanone by chromic acid gave plots of this nature.

From Equation (21),

$$1/k_2 = k_K/k_E k + [CrO_3]/k_E. \qquad (22)$$

Therefore, a plot of $1/k_2$ versus $[CrO_3]$ should be linear. This was found to be the case for the ketones mentioned above.

Now, if the oxidation of glycolaldehyde occurs by the mechanism,

$$\text{Aldehyde} \underset{k_A}{\overset{k_E}{\rightleftharpoons}} \text{Enediol} \xrightarrow[k]{Fe^{+3}} \text{Product},$$

kinetic data should give plots of k_1 and $1/k_2$ versus $[Fe^{+3}]$ similar to those just described for chromic acid oxidation of ketones. Oxidations of 0.01 M glycolaldehyde in 0.25 M $HClO_4$ were carried out at 60°C. with initial ferric ion concentrations

varying from 1 x 10^{-3} M to 0.05 x 10^{-3} M. The results of these oxidations are shown in Fig. 38 and 39. Values of k_1 and k_2 were calculated from initial rates (obtained by drawing tangents to the reaction curves at zero time). A plot of $1/k_2$ against $[Fe^{+3}]$ (both evaluated at zero time) is shown by Fig. 40 to be linear, confirming that the kinetics of ferric ion oxidation of glycolaldehyde are analogous to those described in Equation (17). The plot of k_1 versus $[Fe^{+3}]$ (Fig. 41) has a similar shape to that reported for chromic acid oxidation of ketones.

The data used to plot Fig. 40 and 41 were obtained from a series of runs with varying initial ferric ion concentrations. But, in the case of a reaction in which the substrate is in excess and no secondary reaction or products occurs, the same plots should be obtainable from a single run by determining instantaneous rates at various ferric ion concentrations as the reaction progresses.

This was done for the reaction shown in Fig. 38 whose initial ferric ion concentration was 1 x 10^{-3} Figure 42 gives the $1/k_2$ versus Fe^{+3} plot obtained from this run. The data fit a straight line, and the slope agrees well with that obtained from a series of runs. The slope is the reciprocal of the enolization rate constant and has a reasonable value compared with rates of enolization of other compounds (25).*

Thomas, Trudel, and Bywater (15) found that oxidations of acetoin by 2 x 10^{-4} M ferric perchlorate followed pseudo-first-order kinetics (first-order with respect to ferric ion) in the acidity range from 1.0M to 0.03M $HClO_4$. But when the initial ferric ion concentration was increased to 2 x 10^{-} M, the reaction ceased to obey pseudo-

* An alternate explanation for the effects of acid concentration and initial ferric ion concentration is that dehydration of the aldehyde hydrate is rate-controlling under certain conditions (a zero-order dependence upon the ferric ion concentration could be observed if this were the case). But data in the literature (25) indicate that dehydration of aldehydes is a much faster reaction than the oxidations studied here. Therefore, this explanation may be rejected.

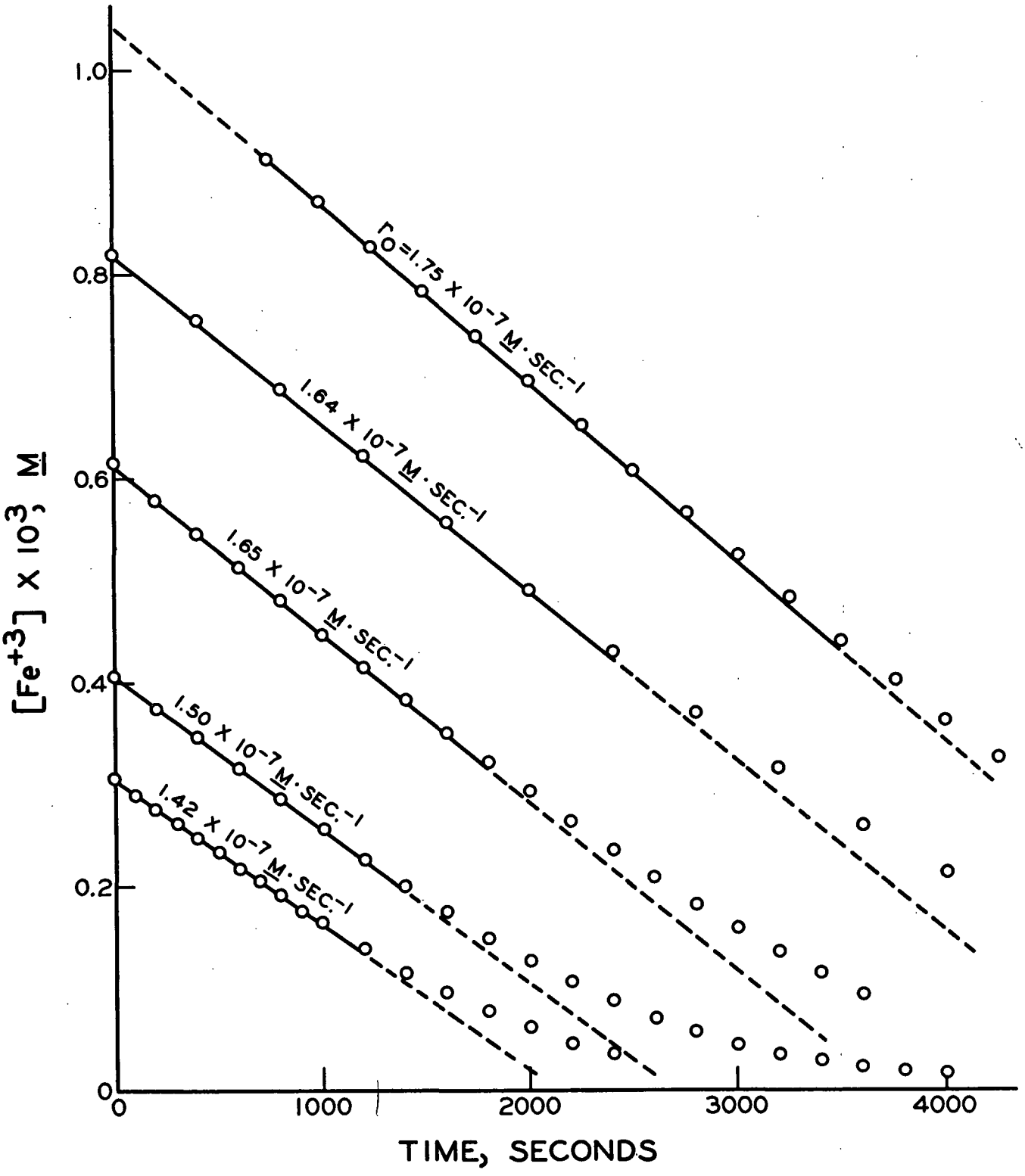


Figure 38. The Effect of the Initial Ferric Ion Concentration on the Initial Rate of Oxidation of 0.010M Glycolaldehyde in 0.250M $HClO_4$ at 60.0°C.

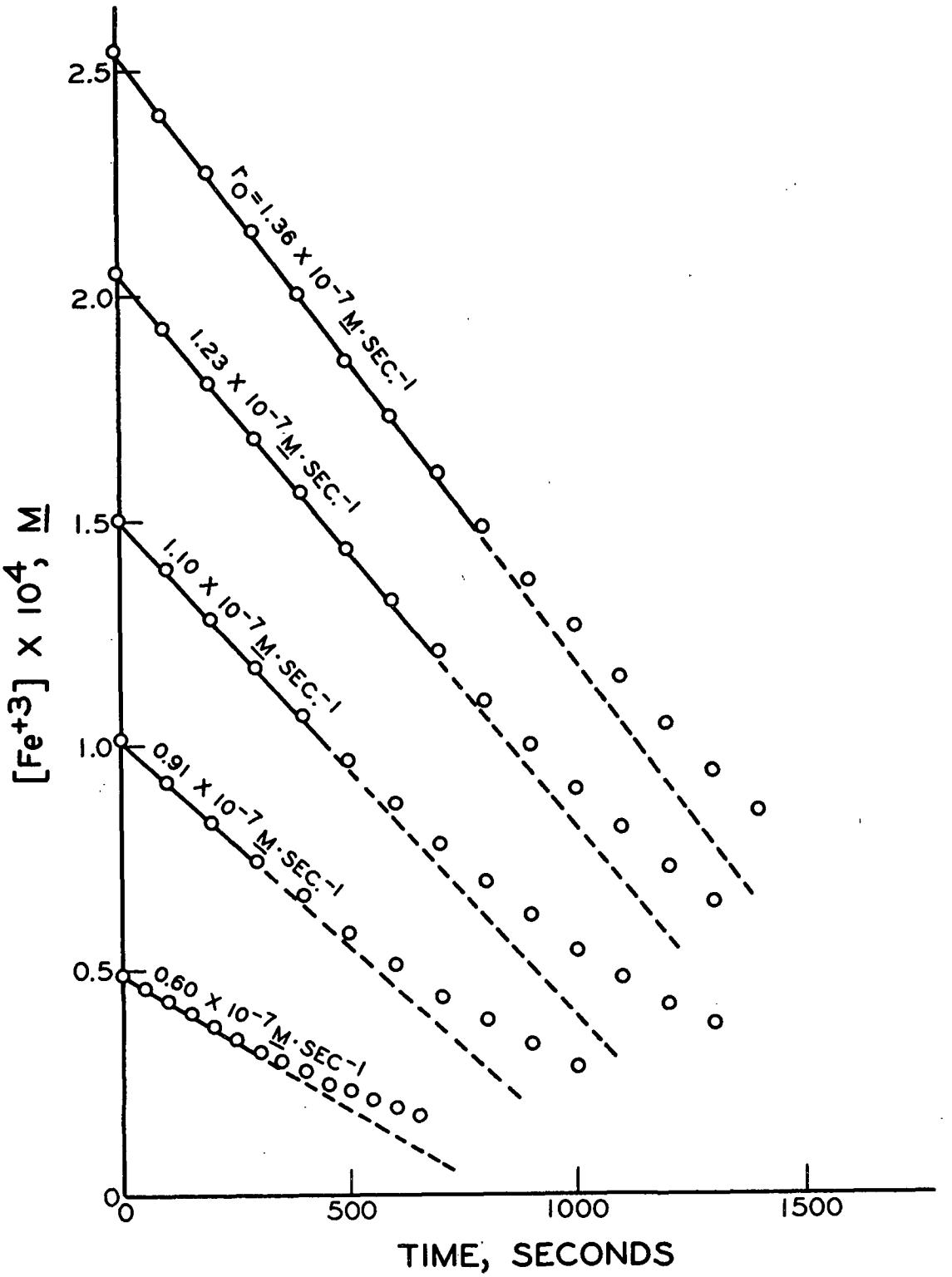


Figure 39. The Effect of the Initial Ferric Ion Concentration on the Initial Rate of Oxidation of 0.0100M Glycolaldehyde in 0.250M $HClO_4$ at 60.0°C.

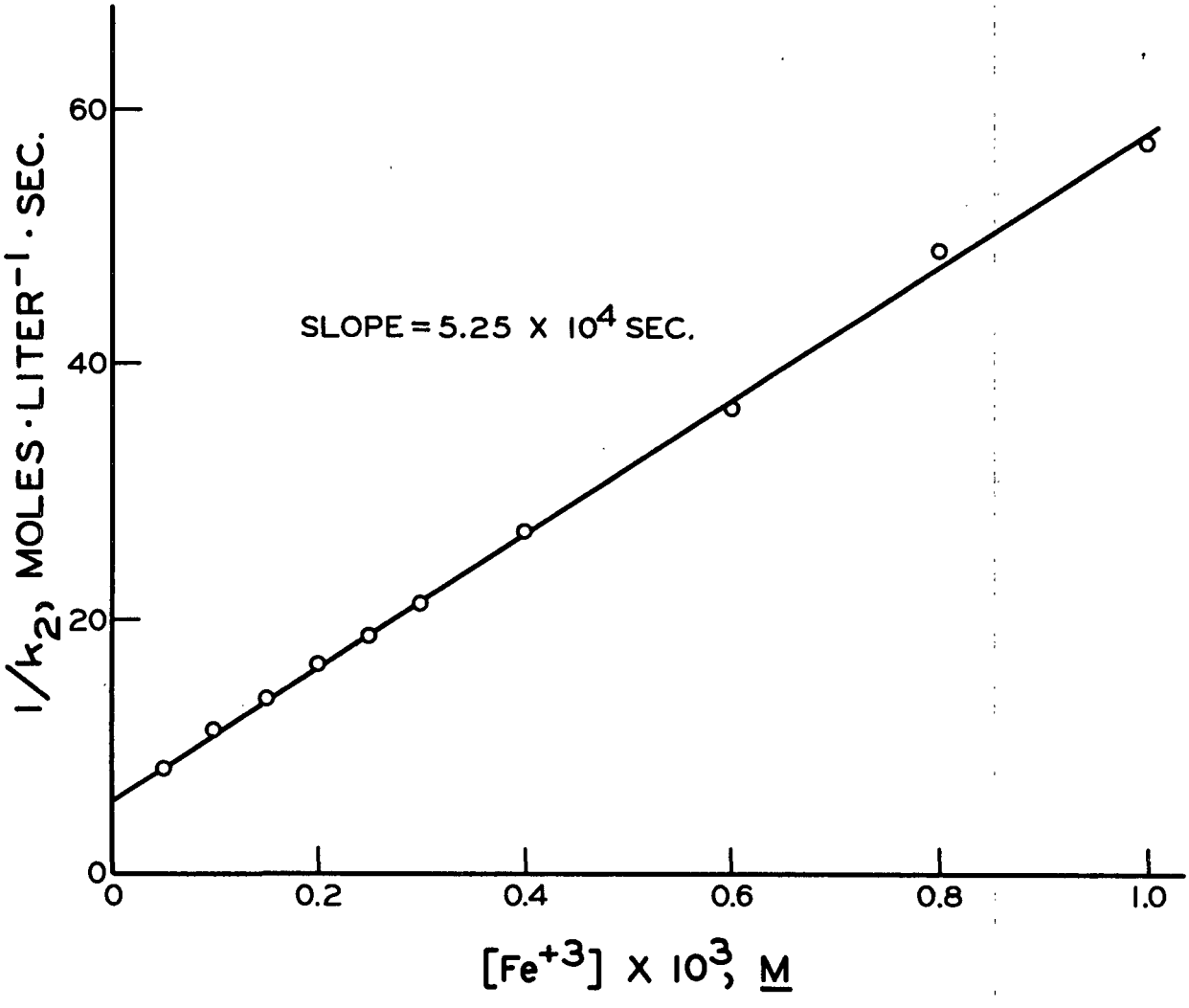


Figure 40. The Relationship Between $1/\underline{k}_2$ and the Initial Ferric Ion Concentration for the Oxidation of 0.0100M Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.250M $HClO_4$ at 60.0°C.

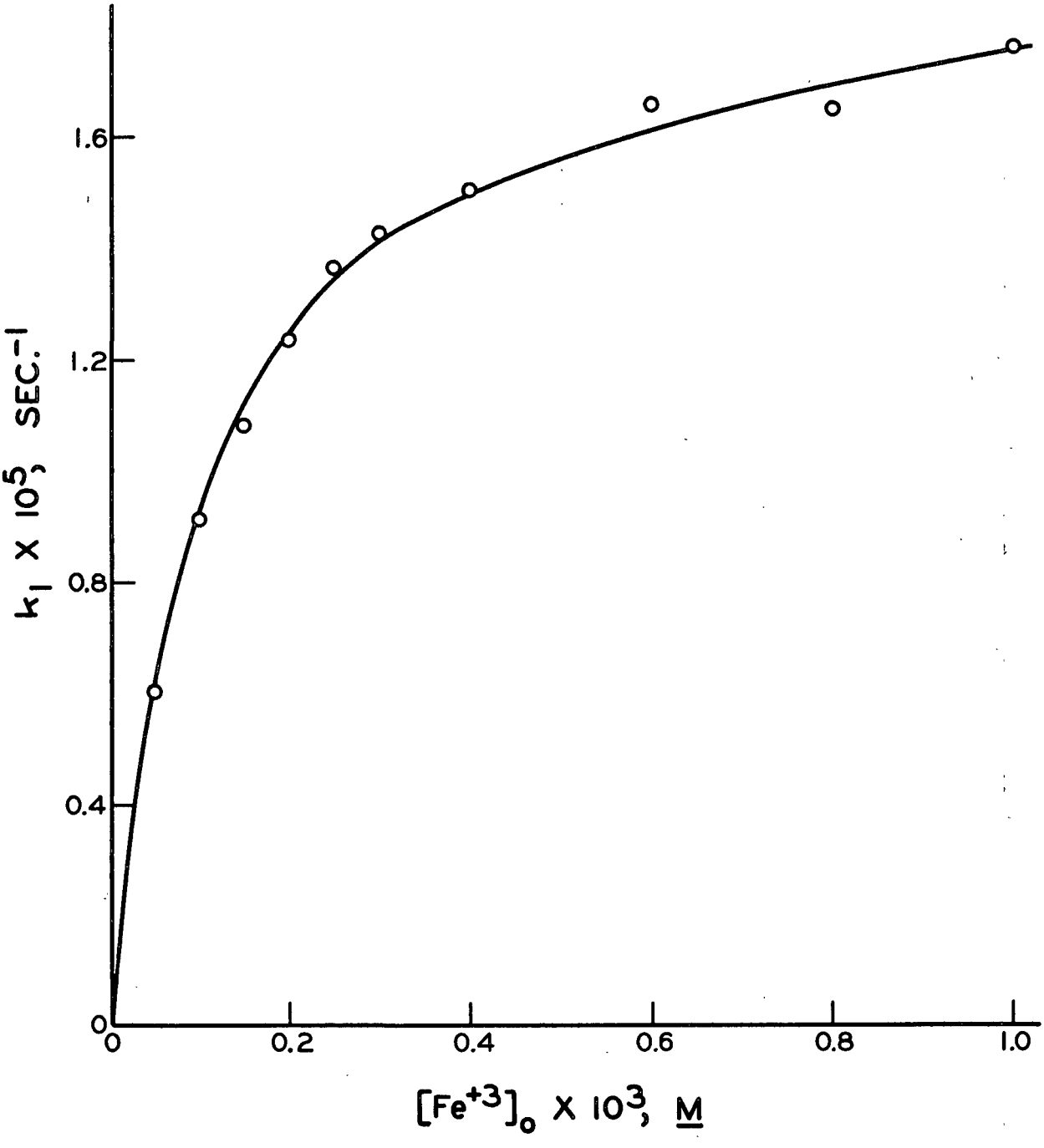


Figure 41. The Relationship Between k_1 and the Initial Ferric Ion Concentration for the Oxidation of 0.0100M Glycolaldehyde in 0.250M $HClO_4$ at 60.0°C.

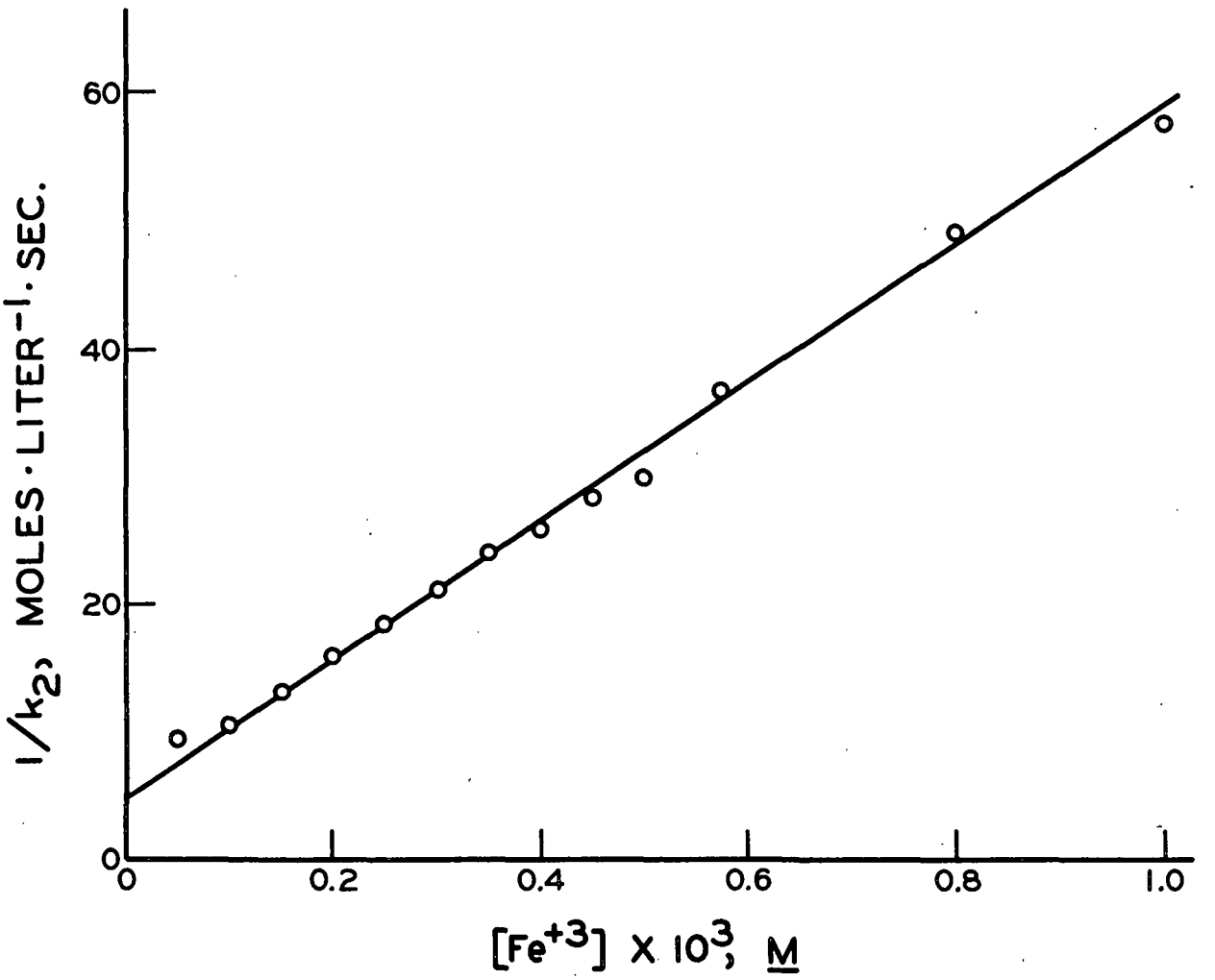


Figure 42. The Relationship Between $1/k_2$ and the Ferric Ion Concentration for the Oxidation of 0.0100M Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.250M $HClO_4$ at 60.0°C. — Data Taken from a Single Run

first-order kinetics at acid concentrations less than about 0.3M. Below this acid concentration, first-order plots were curved over their whole lengths. The initial slopes were less than those observed with 2×10^{-4}M ferric ion, but approached them as the ferric ion concentration dropped.

Departures from pseudo-first-order kinetics seemed to be related to the ratio $[Fe(ClO_4)_3]:[HClO_4]$ since, with 2×10^{-4}M ferric perchlorate, low initial rates were observed at acid concentrations below about 0.03M. The authors suggested that the departures were due to formation of the dimer, $Fe_2(OH)_2^{+4}$, at relatively high ferric ion concentrations and/or low acid concentrations. But they pointed out that the dimerization constant would have to be several powers of ten larger than the accepted value to account for their results.

In view of the evidence which has been obtained for enolization prior to oxidation in the reaction between ferric perchlorate and glycolaldehyde, a more satisfactory explanation can be offered for the deviations from simple kinetics in acetoin oxidations. Assume that the mechanism of oxidation of acetoin involves enolization to an enediol, followed by oxidation of the latter species. Under the conditions in which pseudo-first-order kinetics are followed, oxidation of the enediol is the slow, rate-controlling step. But increasing the ferric ion concentration and/or decreasing the acid concentration would increase the rate of the oxidation step relative to the rate of the enolization step. If this were carried far enough, the oxidation step would no longer be slow enough to be rate-controlling, and a departure from pseudo-first-order kinetics would result.

Thus, oxidations of both glycolaldehyde and acetoin are consistent with the hypothesis that the enediol is the species attacked by ferric ion. This is logical in view of the known high degree of reactivity of enediols toward ferric ion (12). Enolization is probably involved in oxidations of sugars also, since the α-hydroxyaldehyde grouping seems to be responsible for the reactivity of glucose in ferric ion oxidations.

Dependence Upon Reactant Concentrations

It has already been shown that oxidations of 0.01M glycolaldehyde by 0.001M ferric perchlorate at 70.0°C. are zero-order with respect to ferric ion concentration in 0.1M $HClO_4$ and first-order in 1M $HClO_4$. Figures 43 and 44 show the effects that substrate concentration have upon reaction rates in these media.

In Fig. 45 and 46 the rate constants are plotted against glycolaldehyde concentration. In both 0.1M and 1M $HClO_4$ the rate of reaction is directly proportional to the glycolaldehyde concentration; in other words, the dependence upon glycolaldehyde concentration is first-order. This finding is consistent with the mechanism which has been proposed for the oxidation of glycolaldehyde, since both the enolization and oxidation steps would be expected to have a first-order dependence upon substrate concentration.

The Effect of Temperature

The effect of temperature was studied for both the pseudo-zero- and speudo-first-order reactions in 0.1M and 1M $HClO_4$, respectively. Figures 47-50 give the results which were obtained. In both media, rate constants gave reasonably good Arrhenius plots. The calculated activation energies for reactions in 0.1M $HClO_4$ and 1M $HClO_4$, respectively, are 29.3 kcal./mole and 34.2 kcal./mole.

Product Analysis

The expected primary oxidation product from glycolaldehyde is glyoxal, although some glycolic acid might be produced from oxidation of the aldehyde function. It has been shown that 2-aminobenzenethiol and glyoxal react to give a blue-colored product in acid solution (65). The reaction is very specific for glyoxal since glycolaldehyde, pyruvaldehyde, biacetyl, acetaldehyde, and formaldehyde have been found not to interfere.

This reagent was used to determine glyoxal in glycolaldehyde reaction mixtures. Glycolaldehyde and glycolic acid were tested for color formation with

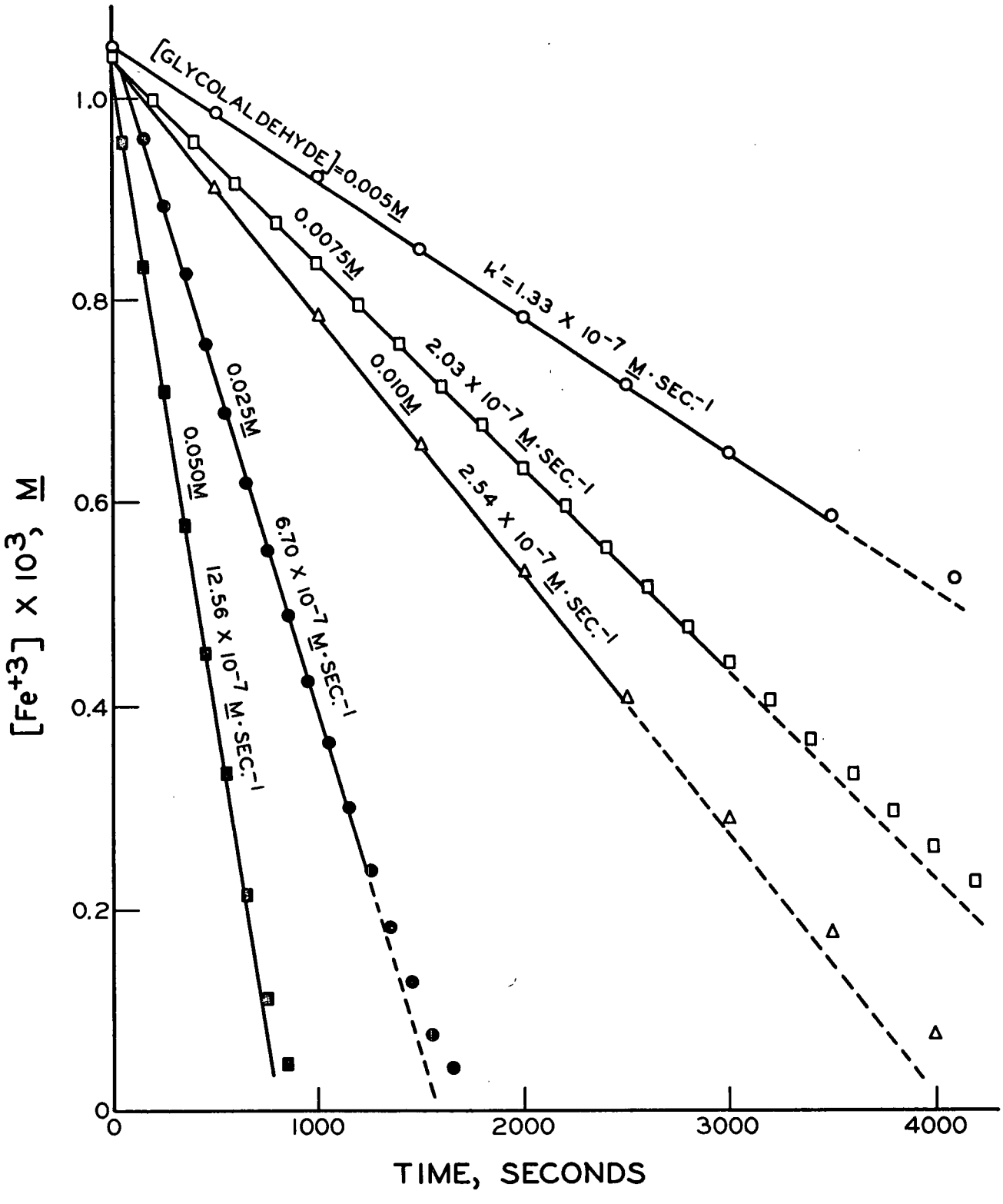


Figure 43. The Effect of Substrate Concentration on the Oxidation of Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.100M $HClO_4$ at 70.0°C.

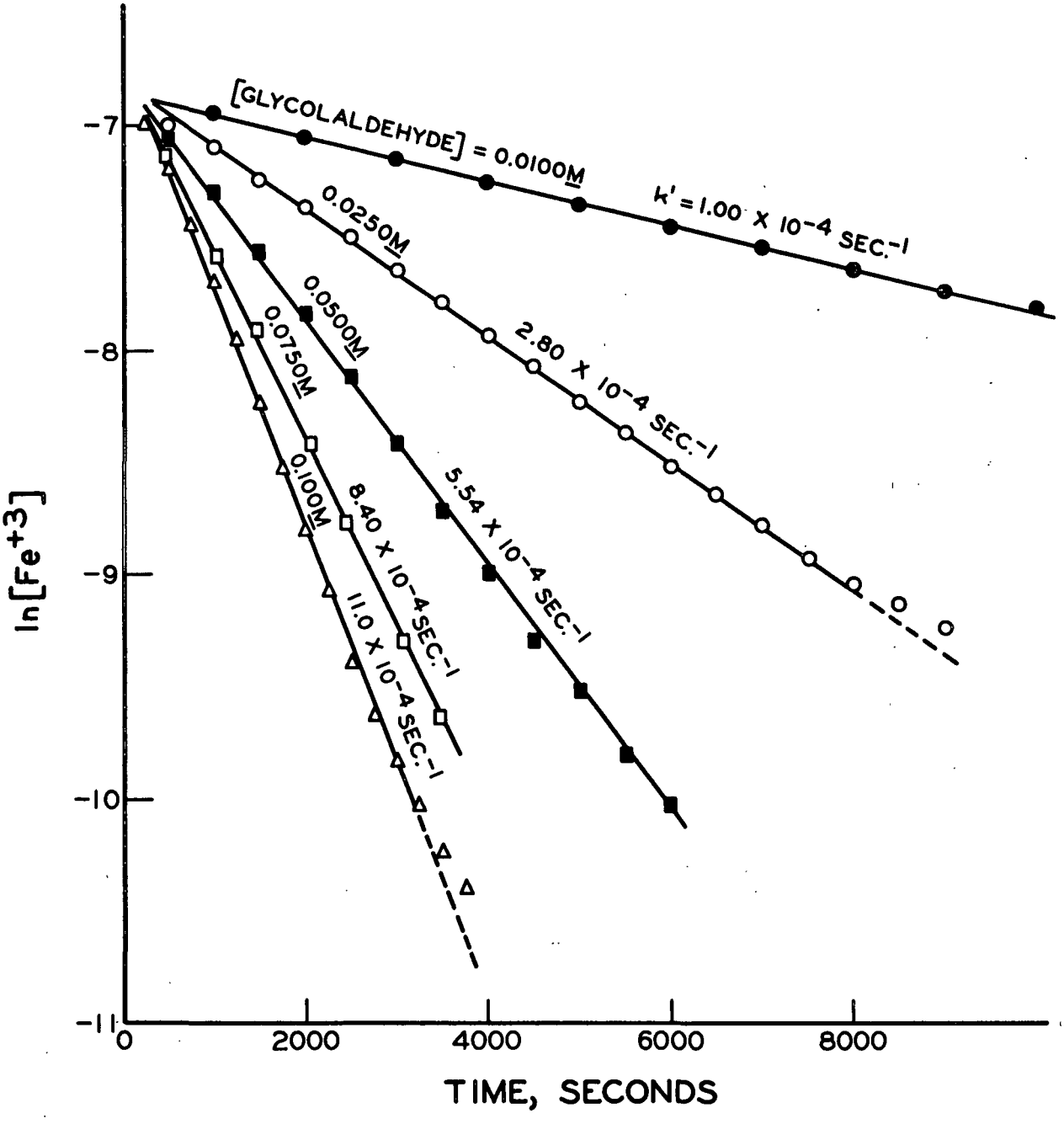


Figure 44. The Effect of Substrate Concentration on the Oxidation of Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 1.00M $HClO_4$ at 50.0°C.

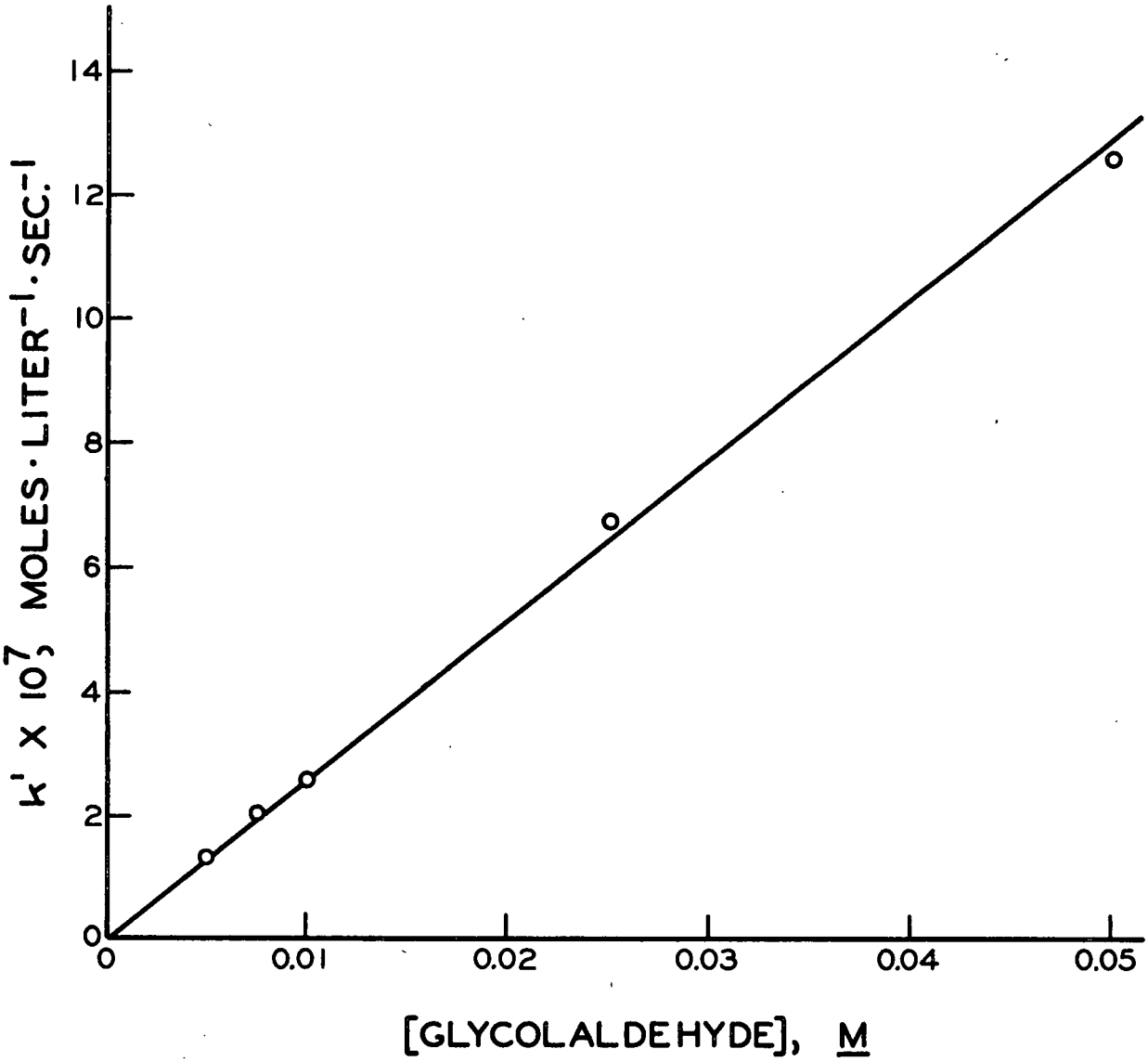


Figure 45. The Relationship Between Substrate Concentration and Reaction Rate Constant for the Oxidation of Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.100M $HClO_4$ at 70.0°C.

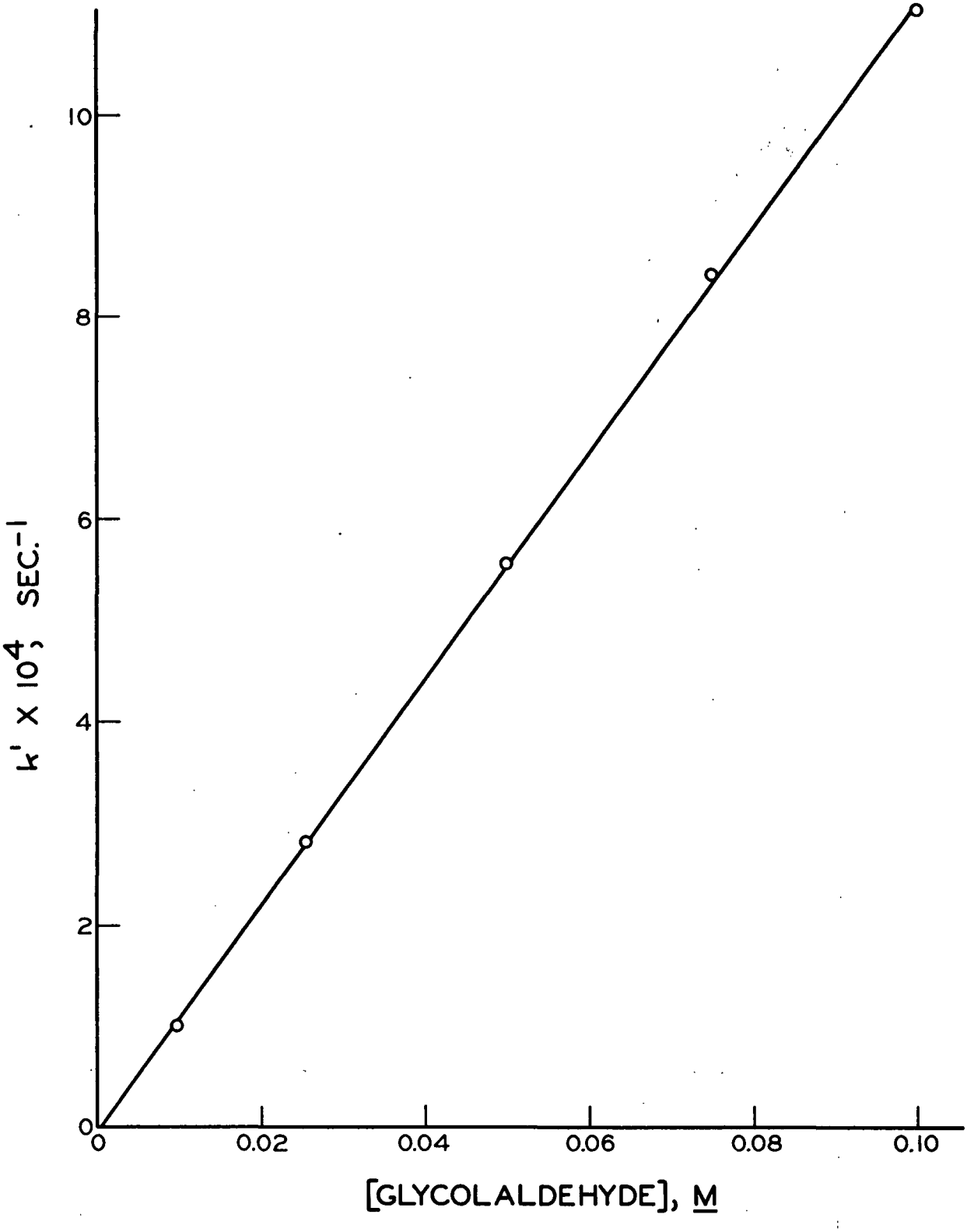


Figure 46. The Relationship Between Substrate Concentration and Reaction Rate Constant for the Oxidation of Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 1.00M $HClO_4$ at 50.0°C.

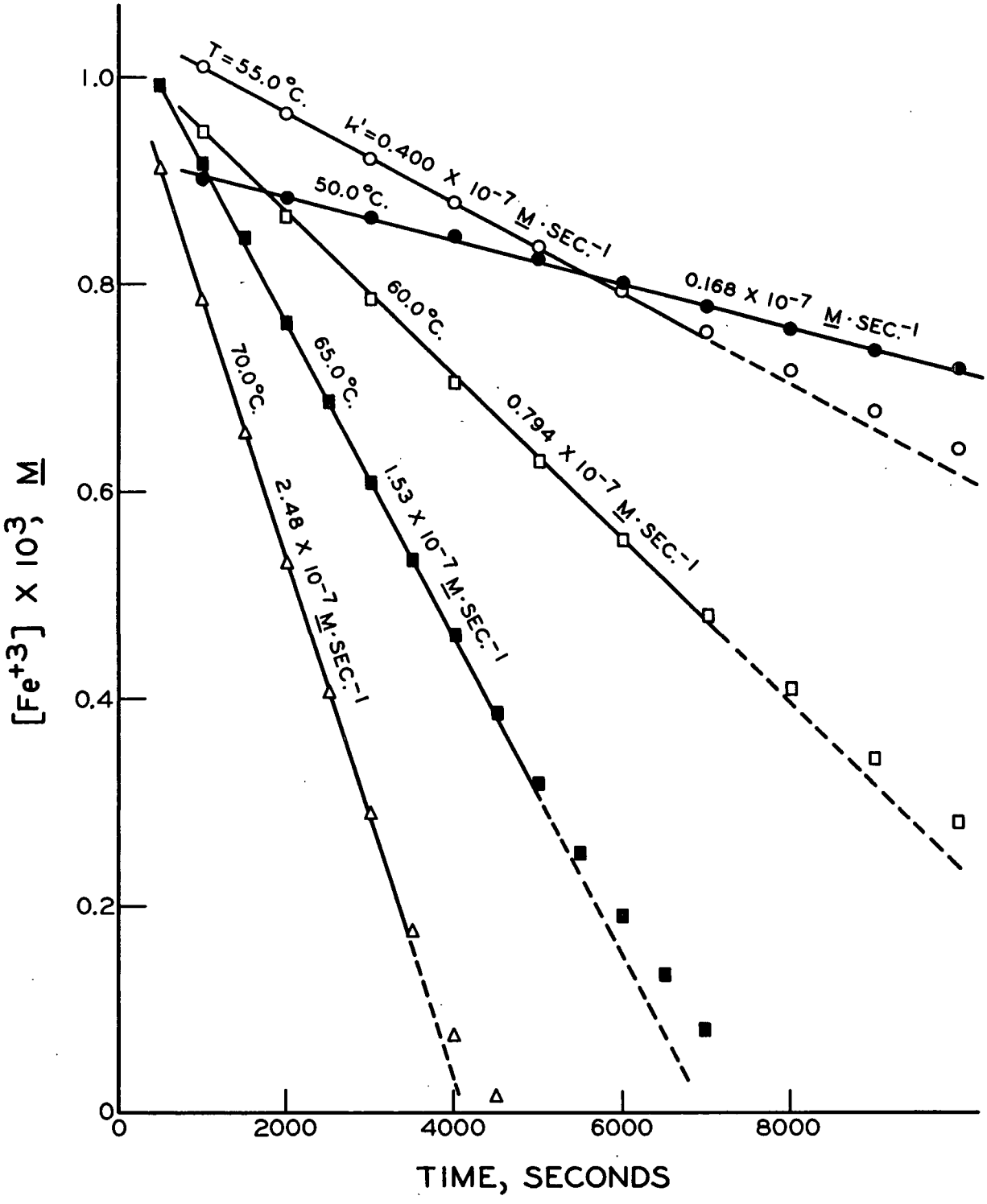


Figure 47. The Effect of Temperature on the Oxidation of 0.0100M Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.100M $HClO_4$

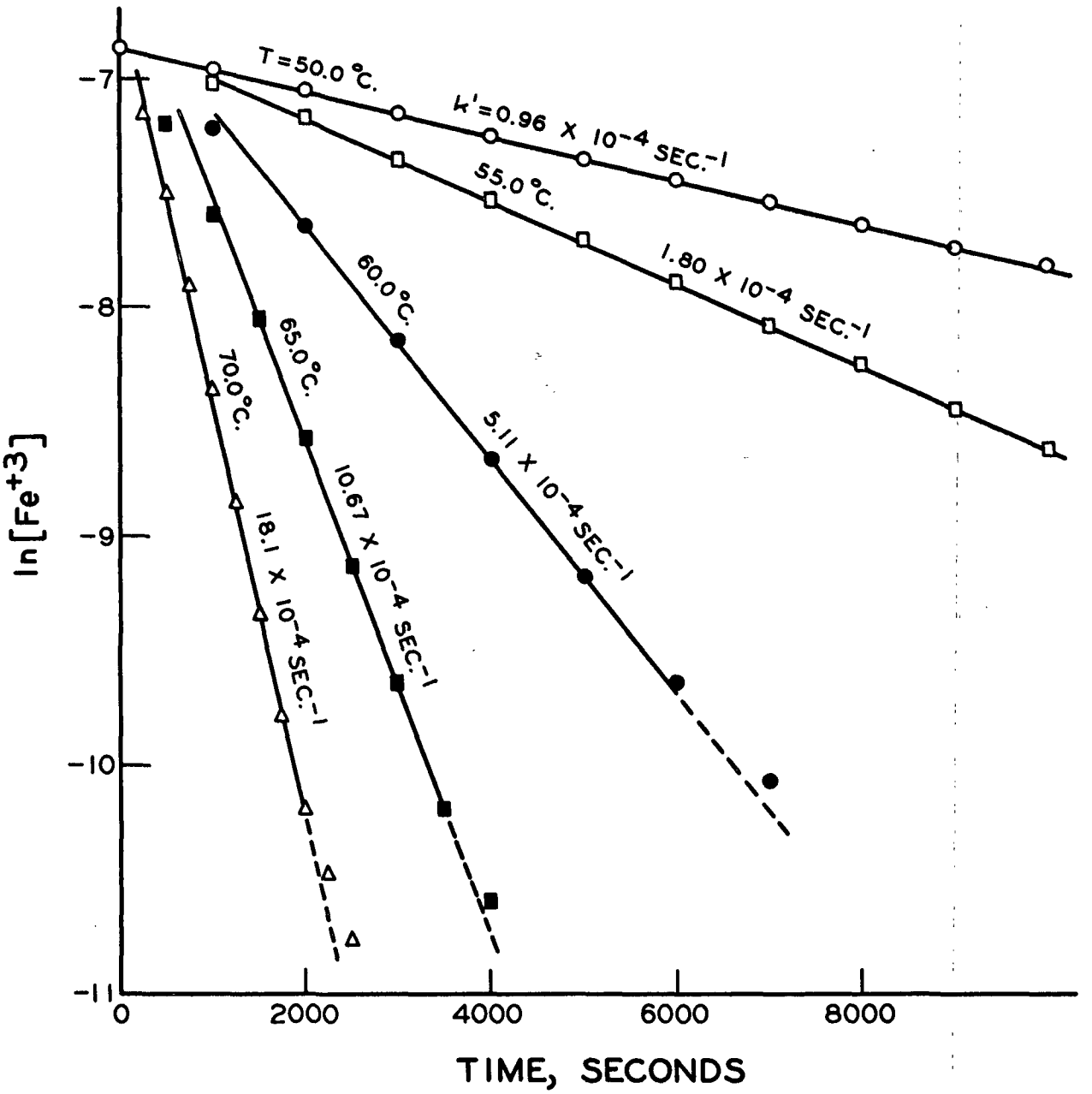


Figure 48. The Effect of Temperature on the Oxidation of 0.0100M Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 1.00M $HClO_4$

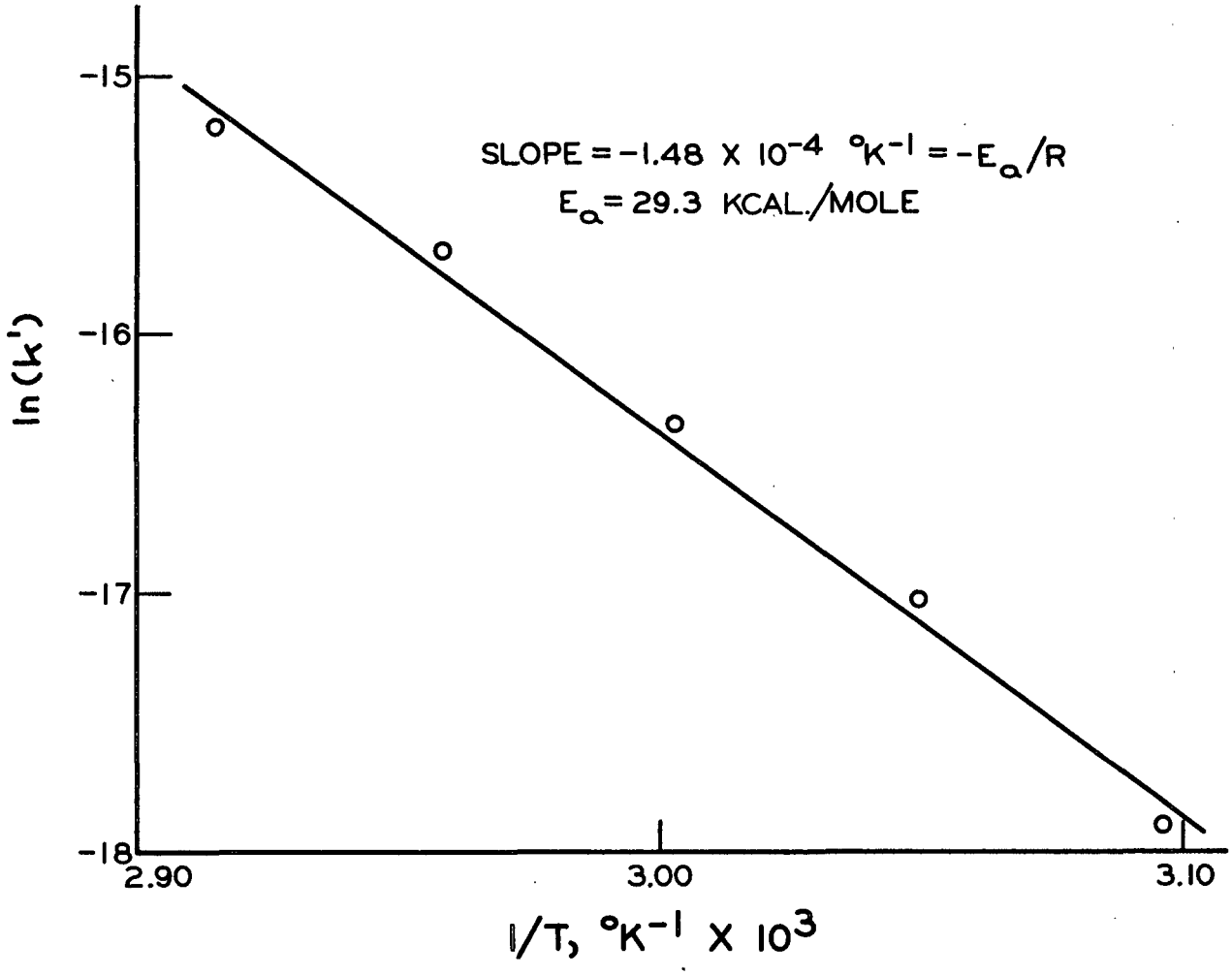


Figure 49. The Arrhenius Plot for the Oxidation of 0.0100M Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.100M $HClO_4$

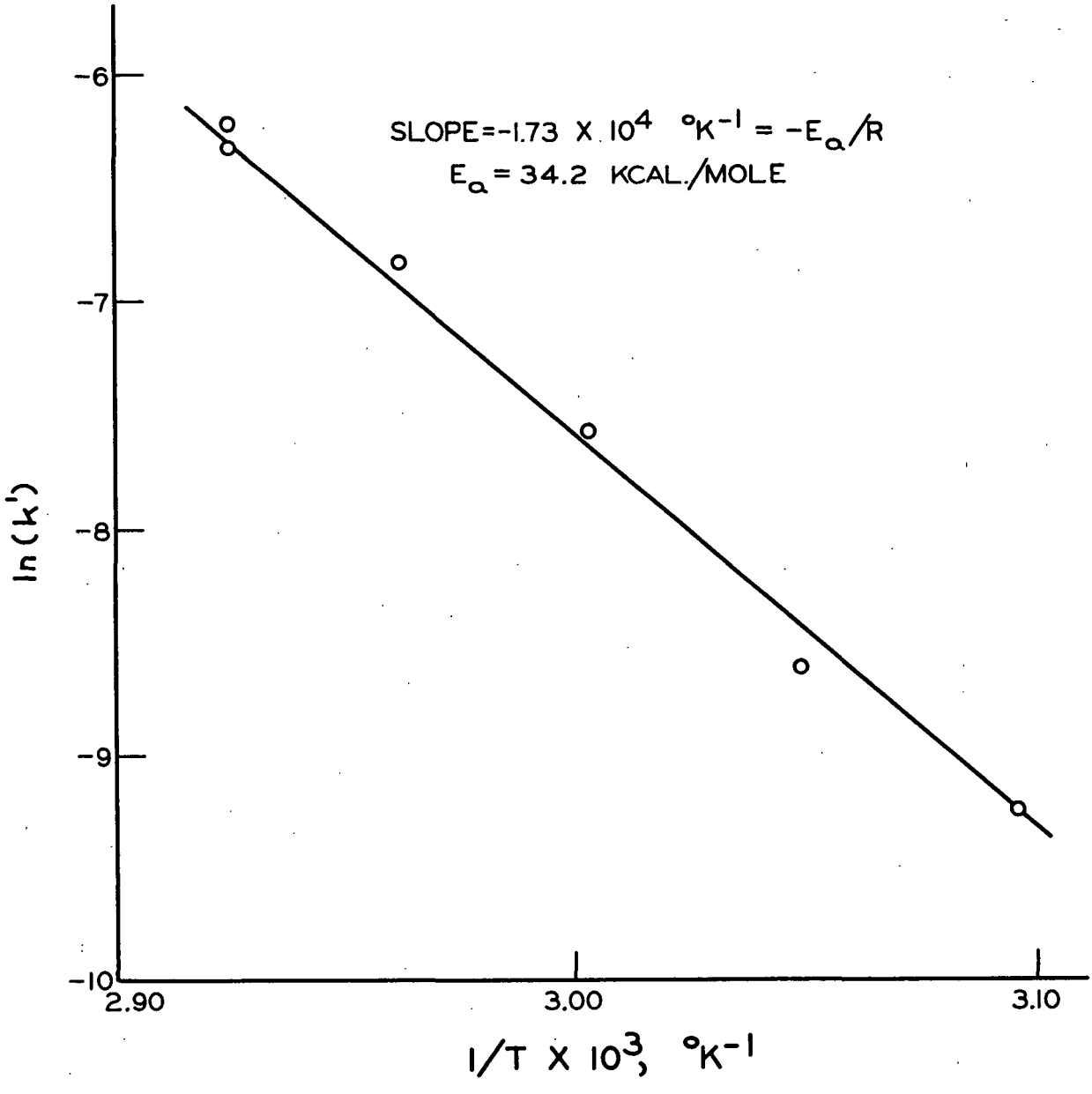


Figure 50. The Arrhenius Plot for the Oxidation of 0.0100M Glycolaldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 1.00M $HClO_4$

2-aminobenzenethiol and the results were negative. It was also shown that the former compound does not interfere with the color reaction between glyoxal and 2-aminobenzenethiol.

The reaction conditions and calibration method were modified from those used previously. Details of the colorimetric procedure are given in Appendix V. The results for two oxidations of 0.01M glycolaldehyde by 0.01M ferric perchlorate at 70°C., one carried out in 0.1M $HClO_4$ and the other in 1M $HClO_4$, are also given in Appendix V.

The results indicate that yields of glyoxal were 87 and 103% of the theoretical yield in 0.1M $HClO_4$ and 1M $HClO_4$, respectively.

Thus, conversion of glycolaldehyde to glyoxal by ferric ion was quantitative in 1M $HClO_4$, and nearly quantitative in 0.1M $HClO_4$. The yield of glyoxal in 0.1M $HClO_4$ may have been higher in kinetic runs since a higher substrate to oxidant ratio was used. This would decrease the likelihood of secondary oxidation of glyoxal.

Proposed Reaction Mechanism

As already discussed, oxidations of glycolaldehyde by ferric perchlorate appear to involve enolization followed by oxidation of the enediol. The kinetics do not indicate that complexing is involved in the oxidation step since glycolaldehyde oxidations exhibit a simple first-order dependence upon substrate concentration, not "Duke's kinetics" (see p. 48). This does not rule out the possibility of oxidation *via* complex formation, however, because there are two conditions under which oxidations involving an intermediate complex would follow first-order kinetics with respect to the substrate concentration. The first is when complex formation, not complex disproportionation, is the rate-controlling step. The

second is when the equilibrium constant for complex formation is small so that $K[S] << 1$. In the latter case, the equation relating the pseudo-first-order rate constant to the substrate concentration,

$$k' = kK[S]/(1 + K[S]),$$

reduces to

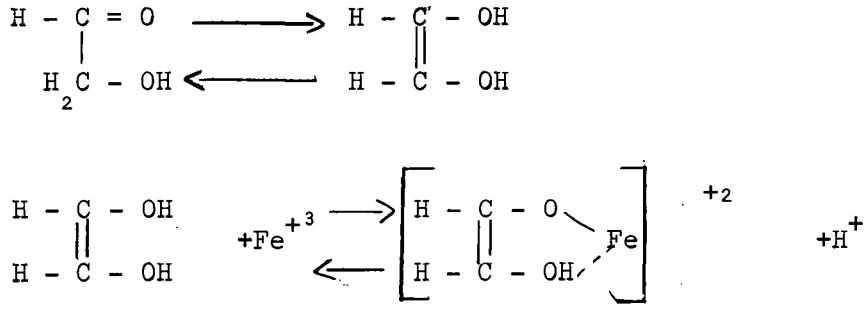
$$k' = kK[S],$$

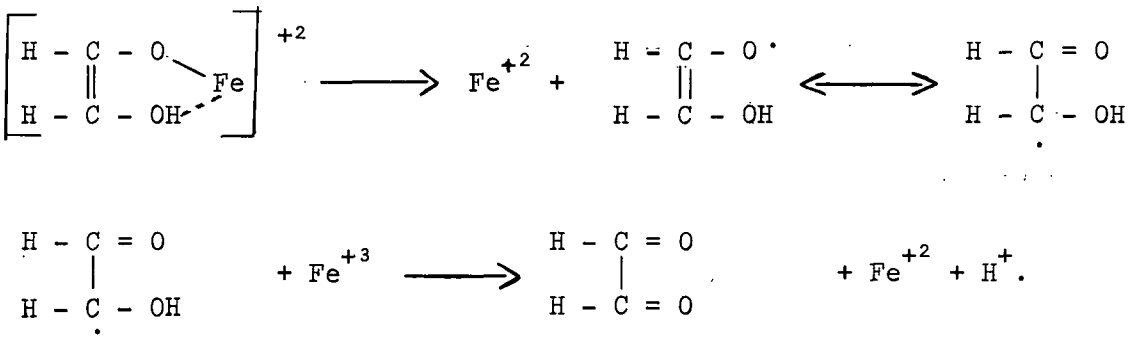
which predicts a first-order dependence upon substrate concentration.

It is felt that complexing is probably involved in the mechanism of oxidation of glycolaldehyde by ferric perchlorate for the following reasons:

1. The enediol appears to be the species which is attacked by ferric ion, and complexing has been shown to be involved in ferric ion oxidations of enediols (12).
2. Glycolaldehyde has been shown to complex with ferric ion (p. 20).
3. There is evidence which indicates that glucose and 3-O-methylglucose are oxidized via complex formation. These sugars are thought to be oxidized by the same mechanism as for glycolaldehyde. Therefore, oxidation of glycolaldehyde should also involve complex formation.

The proposed reaction mechanism for the ferric ion oxidation of glycolaldehyde is as follows:





GLYCERALDEHYDE

The oxidation of glyceraldehyde was studied in order to determine whether an α,β-dihydroxyaldehyde behaves differently from an α-hydroxyaldehyde. In addition to exerting inductive, steric, and hydrogen-bonding effects, the additional hydroxyl might affect the reaction mechanism because of its potential for complexing ferric ion. Effects of the β-hydroxyl and other hydroxyl groups are of interest because of the polyhydroxylic nature of glucose.

Kinetics

Oxidations of 0.01M glyceraldehyde by 10^{-3}M ferric perchlorate at 70.0°C. are shown in Fig. 51 at a number of acid concentrations (constant molar ionic strength of 1.0). The results show that, unlike glycolaldehyde, the rate of oxidation of glyceraldehyde falls off continuously as the acid concentration is increased. And, except for a low initial rate in 0.01M $HClO_4$, oxidations of glyceraldehyde follow first-order kinetics with respect to ferric ion at all acidities which were studied.

If the mechanism of oxidation of glyceraldehyde is the same as that which has been proposed for glycolaldehyde, the constant adherence to pseudo-first-order kinetics in the case of the former compound implies that the oxidation step is rate-controlling over the range of acid concentrations from 0.01M to 0.25M. This being the case, the observed dependence of reaction rate upon acid concentration ought to be consistent with the proposed mechanism.

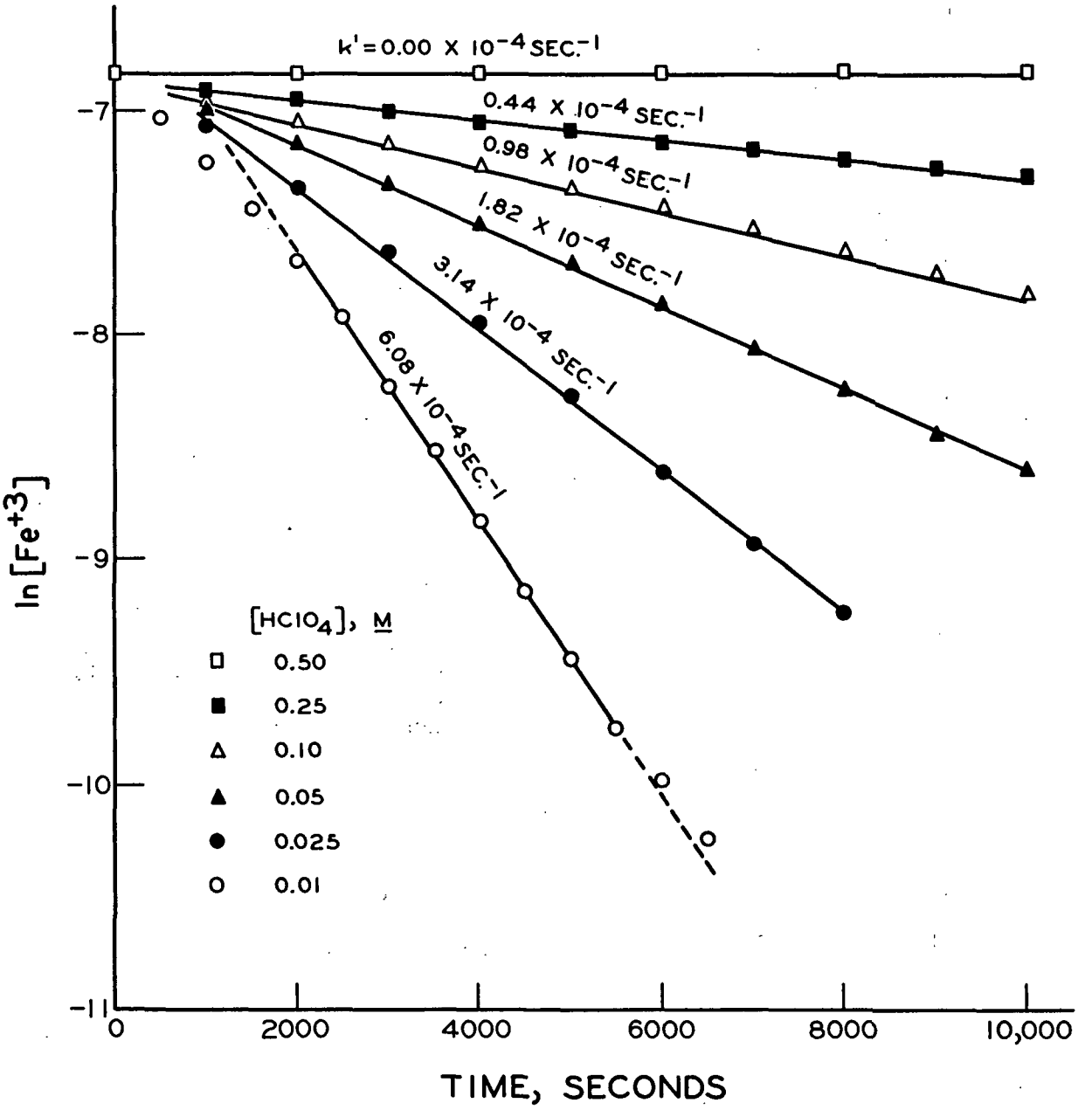


Figure 51. The Effect of Acid Concentration on the Oxidation of 0.0100M Glyceraldehyde by 10^{-3}M $Fe(ClO_4)_3$ at 70.0°C.

From a consideration of the proposed reaction mechanism, it appears that the acid concentration could affect the overall rate of oxidation of glyceraldehyde through its effect upon the ferric ion hydrolysis and complex formation equilibria.

The ferric ion hydrolysis equilibrium,

$$Fe^{+3}_{aq} + H_2O \overset{K_h}{\rightleftharpoons} FeOH^{+2} + H^+,$$

is acid-dependent because a proton is produced in the hydrolysis reaction. Thus, the relative proportions of the two species, Fe^{+3}_{aq} and $FeOH^{+2}$, depend upon the acid concentration. From Milburn's data (66), the hydrolysis constant for ferric ion is estimated to be 0.0157 at 70.0°C. and an ionic strength of 1.0, from which it can be calculated that 10^{-3}M ferric perchlorate solutions are 1.55, 13.6, and 61.1% in the $FeOH^{+2}$ form in 1M $HClO_4$, 0.1M $HClO_4$, and 0.01M $HClO_4$, respectively, under these conditions.

The reactivities of Fe^{+3}_{aq} and $FeOH^{+2}$ may be expected to differ with respect to their tendencies to complex with and oxidize organic compounds. Thomas, Trudel, and Bywater (15), in their study of acetoin oxidations by ferric perchlorate, concluded that $FeOH^{+2}$ was more reactive than Fe^{+3}_{aq}, but it will be subsequently shown that if complex formation is taken into consideration, the kinetics of acetoin and glyceraldehyde oxidations are consistent with the hypothesis that Fe^{+3}_{aq} is the reactive species.

Formation of the type of complex proposed by Arndt, Loewe, and Ayca (12) between enediols and ferric ion is also an acid-dependent equilibrium because of proton displacement:

$$\text{Enediol} + Fe^{+3} \quad (Fe^{+3}_{aq} \text{ or } FeOH^{+2}) \overset{K_c}{\rightleftharpoons} \text{Complex} + H^+.$$

Thus, the complex concentration is greater the lower the acid concentration.

Consider the system of reactions,

$$\text{Substrate (S)} \underset{}{\overset{K_E}{\rightleftharpoons}} \text{Enediol (E)},$$

$$Fe^{+3}_{aq} + H_2O \overset{K_h}{\rightleftharpoons} FeOH^{+2} + H^+,$$

$$\text{Enediol} + Fe^{+3}_{aq} \overset{K_c}{\rightleftharpoons} \text{Complex (C)} + H^+,$$

$$\text{Complex} \xrightarrow[\text{slow}]{k} \text{Radical} + Fe^{+2}$$

$$\text{Radical} + Fe^{+3}\ (Fe^{+3}_{aq} \text{ or } FeOH^{+2}) \xrightarrow[\text{fast}]{} \text{Product} + Fe^{+2} + H^+.$$

The rate of reduction of the total ferric ion,

$$Fe^{+3}_t = Fe^{+3}_{aq} + FeOH^{+2} + C, \tag{23}$$

is given by the equation

$$-d[Fe^{+3}_t]/dt = d[Fe^{+2}]/dt = 2k[C]. \tag{24}$$

The complex concentration, [C], can be obtained from the equilibrium equation for complex formation:

$$[C] = K_c[E]\ [Fe^{+3}_{aq}]/[H^+]. \tag{25}$$

Substituting into Equation (24) gives

$$-d[Fe^{+3}_t]/dt = 2kK_c[E]\ [Fe^{+3}_{aq}]/[H^+], \tag{26}$$

which may be rewritten in terms of the substrate concentration as

$$-d[Fe^{+3}_t]/dt = 2K_cK_E[S]\ [Fe^{+3}_{aq}]/[H^+]. \tag{27}$$

From Equation (23) and the substitutions,

$$[FeOH^{+2}] = K_h[Fe^{+3}_{aq}]/[H^+] \text{ and} \tag{28}$$

$$[C] = K_cK_E[S]\ [Fe^{+3}_{aq}]/[H^+], \tag{29}$$

the concentration of Fe^{+3}_{aq} may be expressed as a function of the total ferric ion concentration:

$$[Fe^{+3}_{aq}] = [Fe^{+3}_t]/\left\{1 + 1/[H^+](K_h + K_cK_E[S])\right\}. \tag{30}$$

Substitution of Equation (30) into Equation (27) gives the rate expression,

$$-d[Fe^{+3}_t]/dt = \frac{2kK_cK_E[S]}{[H^+] + K_h + K_cK_E[S]} [Fe^{+3}_t]. \quad (31)$$

When $[S] >> [Fe^{+3}_t]$, Equation (31) may be simplified to the pseudo-first-order rate expression,

$$-d[Fe^{+3}_t]/dt = k'[Fe^{+3}_t], \quad (32)$$

in which

$$k' = \frac{2kK_cK_E[S]}{[H^+] + K_h + K_cK_E[S]} . \quad (33)$$

The last equation gives the relationship between the pseudo-first-order rate constant and the acid and substrate concentrations. When $K_cK_E[S] + K_h << [H^+]$, the approximation,

$$k' \approx 2kK_cK_E[S]/[H^+] \quad (34)$$

predicts a linear relationship between k' and $1/[H^+]$. This is the type of acid dependence which was reported for the ferric perchlorate oxidation of acetoin at 40°C. (15). However, as can be seen from Fig. 52, there is a nonlinear relationship between k' and $1/[H^+]$ in the case of oxidations of glyceraldehyde at 70°C. probably a result of the approximation given in Equation (34) failing to hold at this temperature. The hydrolysis constant for ferric ion is estimated from Milburn's data (66) to be 0.0157 at 70°C. as compared with 0.0035 at 40°C. The latter is small in comparison with $[H^+]$, whereas the former is not.

Inversion of Equation (33) gives

$$1/k' = \frac{[H^+]}{2kK_cK_E[S]} + \frac{K_h}{2kK_cK_E[S]} + \frac{1}{2k} , \quad (35)$$

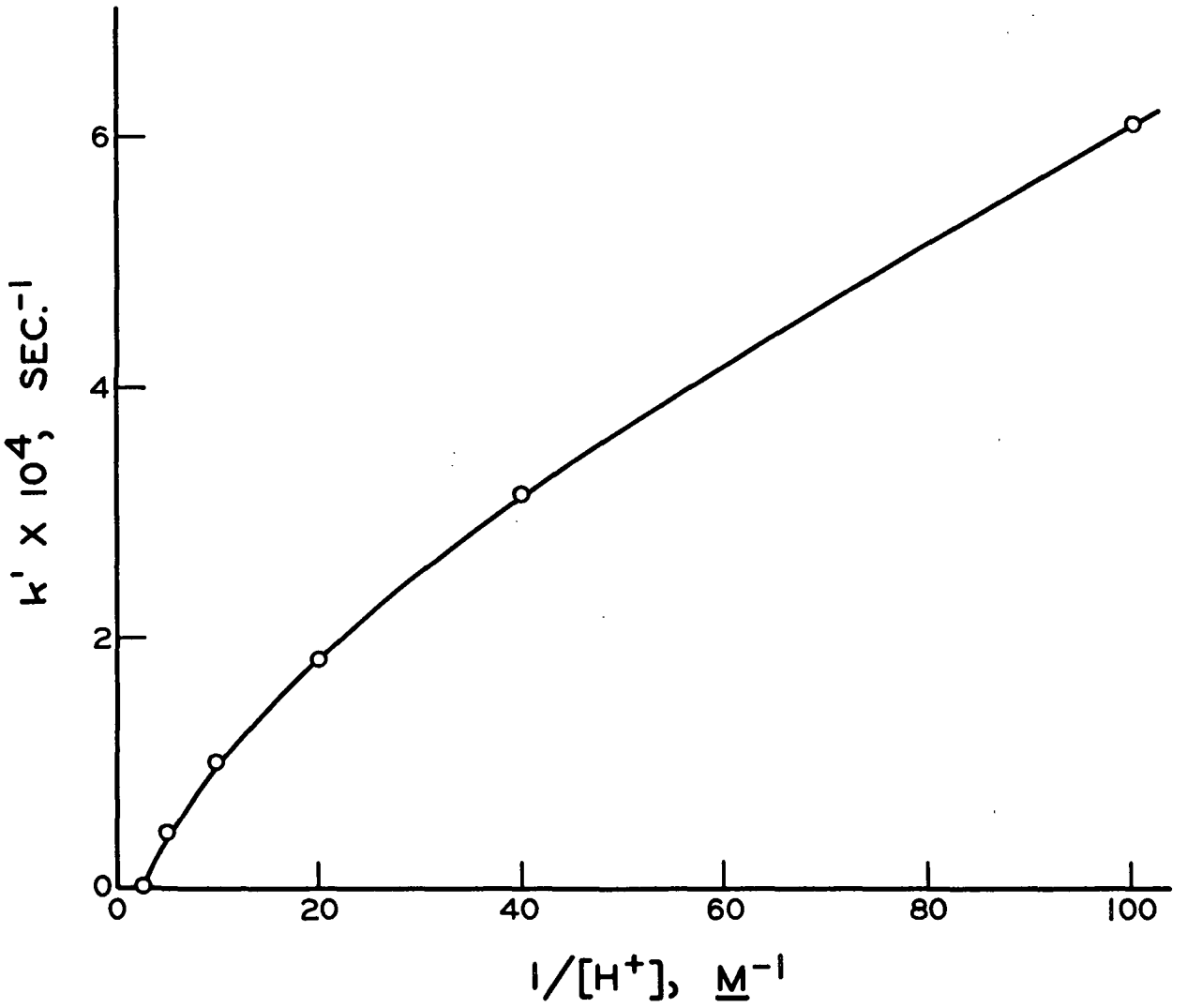


Figure 52. Plot of k' versus $1/[H^+]$ for the Oxidation of 0.0100M Glyceraldehyde by 10^{-3}M $Fe(ClO_4)_3$ at 70.0°C.

which predicts that, at constant [S], $1/\underline{k}'$ will be linearly related to $[H^+]$. This is shown by Fig. 53 to be true for the oxidation of glyceraldehyde.

Equation (33) predicts Duke's kinetics (see p. 48) for the dependence of $\underline{k}'$ upon substrate concentration. But when $\underline{K_c}\underline{K_E}[S] \ll \underline{K_h} + [H^+]$, Equation (33) reduces to

$$k' \approx 2kK_cK_E[S]/([H^+] + K_h), \qquad (36)$$

which implies first-order kinetics with respect to the substrate concentration. This was reported to be the case for ferric perchlorate oxidations of acetoin (15), and the results shown in Fig. 54 and 55 indicate that glyceraldehyde oxidations also have a first-order dependence upon substrate concentration.

It can be seen from Equation (35) that the ratio of the slope of a plot of $1/\underline{k}'$ versus $1/[S]$ to the slope of a plot of $1/\underline{k}'$ versus $[H^+]$ is equal to $([H^+] + \underline{K_h})[S]$. From Fig. 52 and 56, $\underline{K_h}$ is calculated to be 0.011, in good agreement with a value of 0.0157 at 70°C., extrapolated from Milburn's data (66), supporting the validity of the assumed reaction mechanism.

But the kinetics of acetoin and glyceraldehyde oxidations are also consistent with a reaction mechanism which does not involve complex formation. Consider the system,

$$Fe^{+3}_{aq.} + S \xrightarrow{k_1} \text{Radical} + Fe^{+2} + H^+$$

$$FeOH^{+2} + S \xrightarrow{k_2} \text{Radical} + Fe^{+2} + H_2O$$

$$\text{Radical} + Fe^{+3}_{aq} \text{ (or } FeOH^{+2}) \xrightarrow[\text{fast}]{} \text{Product} + Fe^{+2} + H^+ \text{ (or } H_2O)$$

$$Fe^{+3}_{aq} + H_2O \underset{}{\overset{K_h}{\rightleftharpoons}} FeOH^+ + H^+.$$

The rate of reduction of the total ferric ion is given by

$$-d[Fe^{+3}_t]/dt = 2(k_1[Fe^{+3}_{aq}] + k_2[FeOH^{+2}])\,[S], \qquad (37)$$

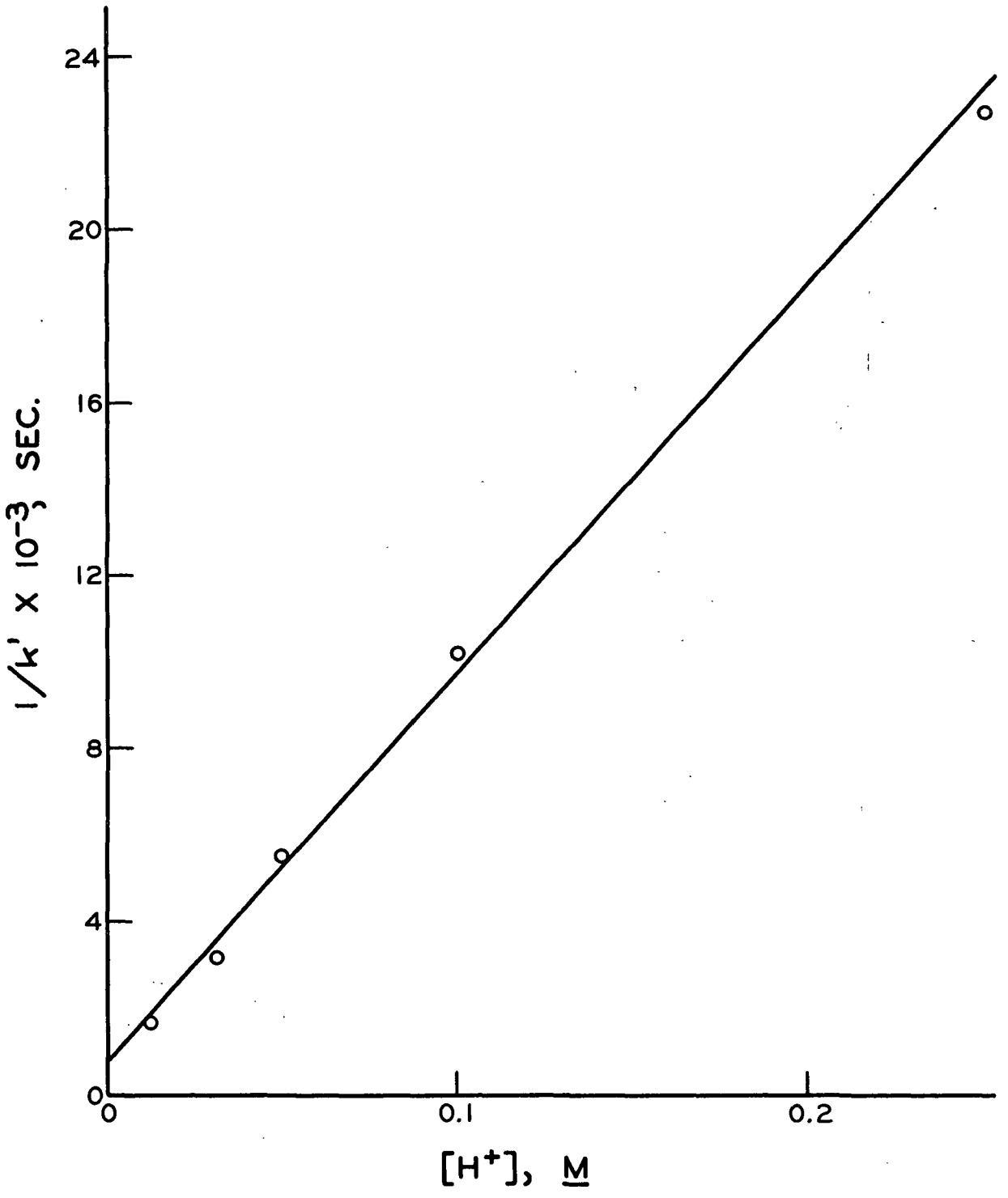


Figure 53. Plot of $1/\underline{k}'$ versus $[\underline{H}^+]$ for the Oxidation of 0.0100$\underline{M}$ Glyceraldehyde by $10^{-3}\underline{M}$ $Fe(ClO_4)_3$ at 70.0°C.

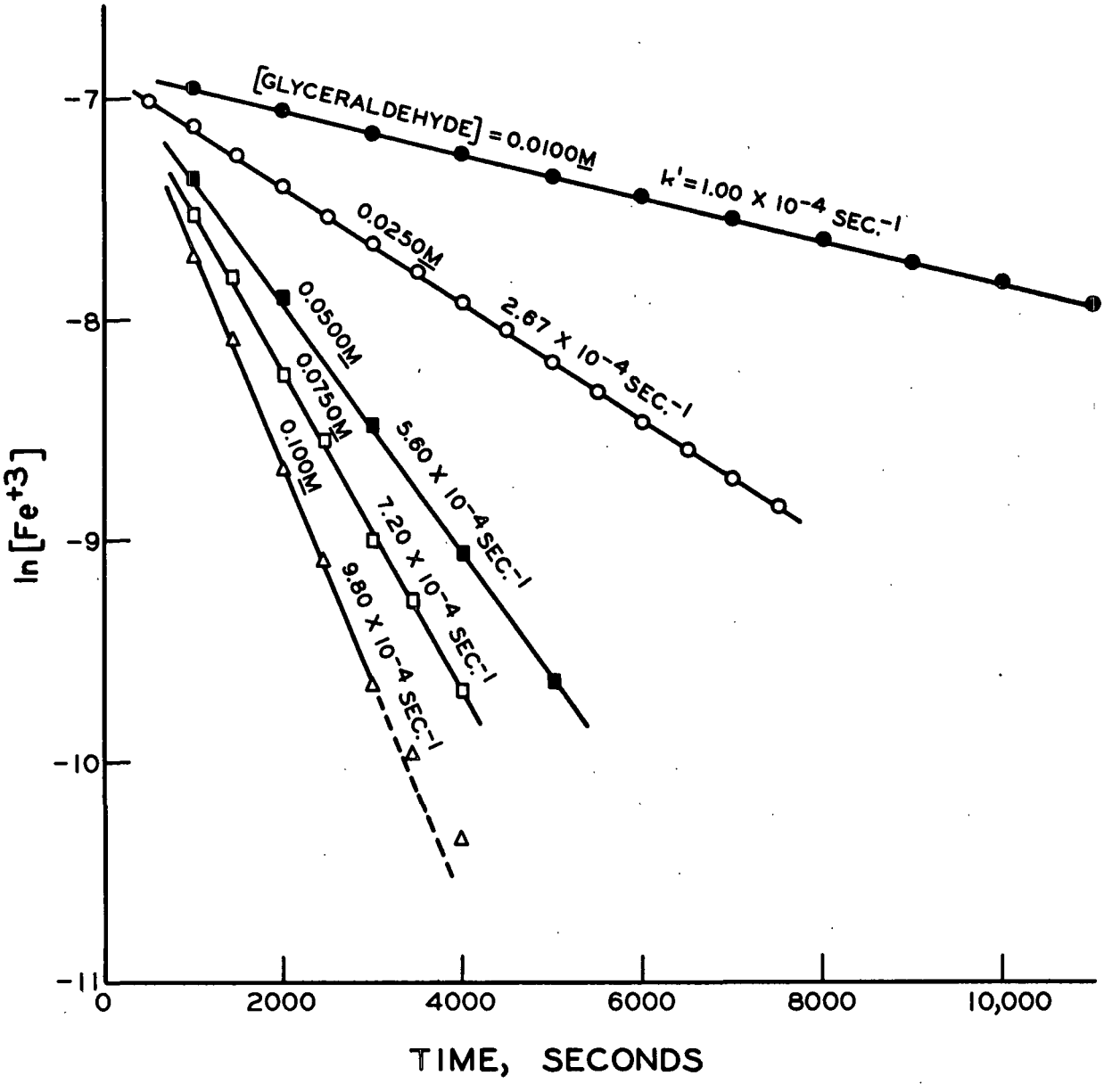


Figure 54. The Effect of Substrate Concentration on the Oxidation of Glyceraldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.100M $HClO_4$ at 70.0°C.

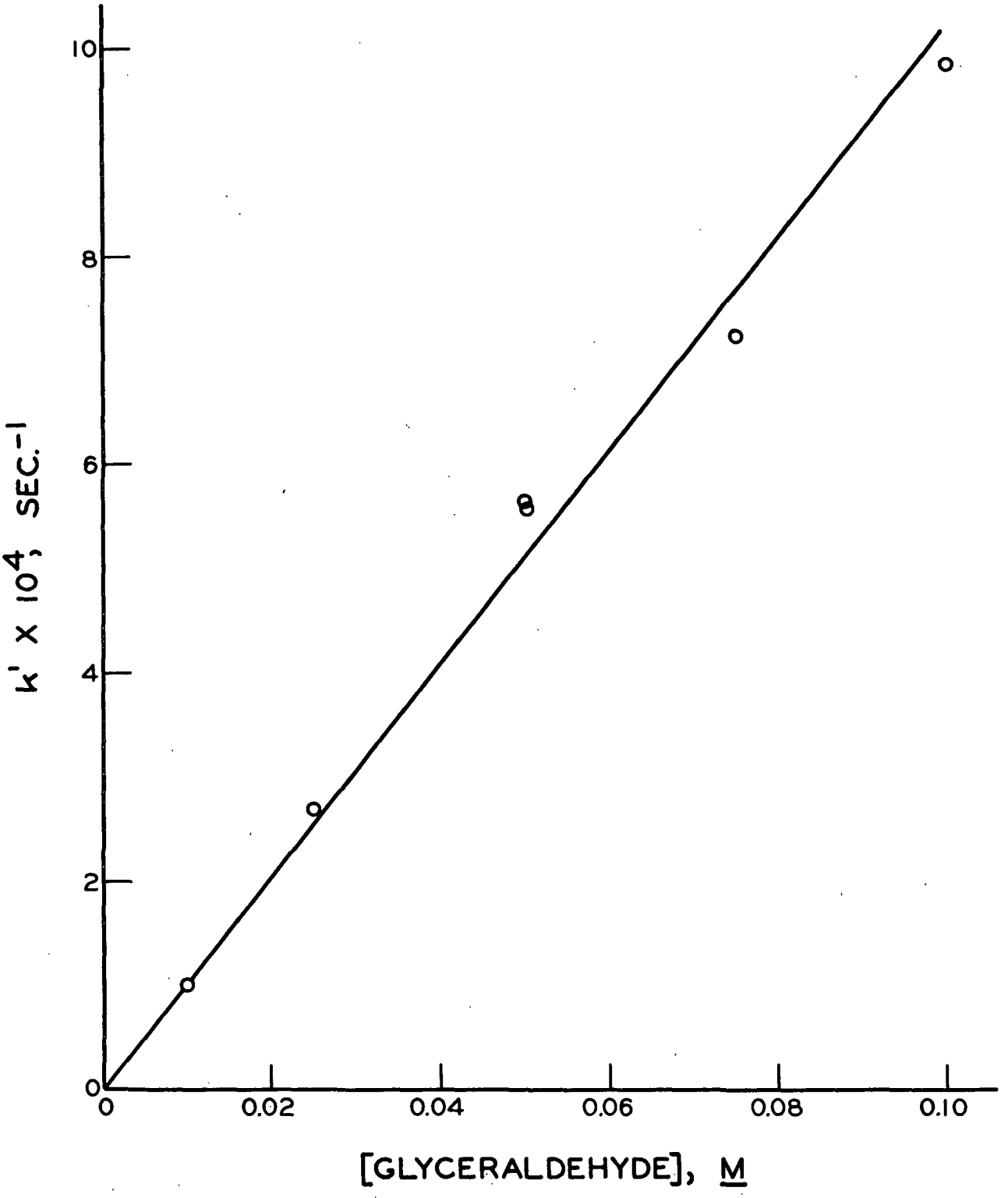


Figure 55. The Relationship Between Substrate Concentration and Reaction Rate Constant for the Oxidation of Glyceraldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.100M $HClO_4$ at 70.0°C.

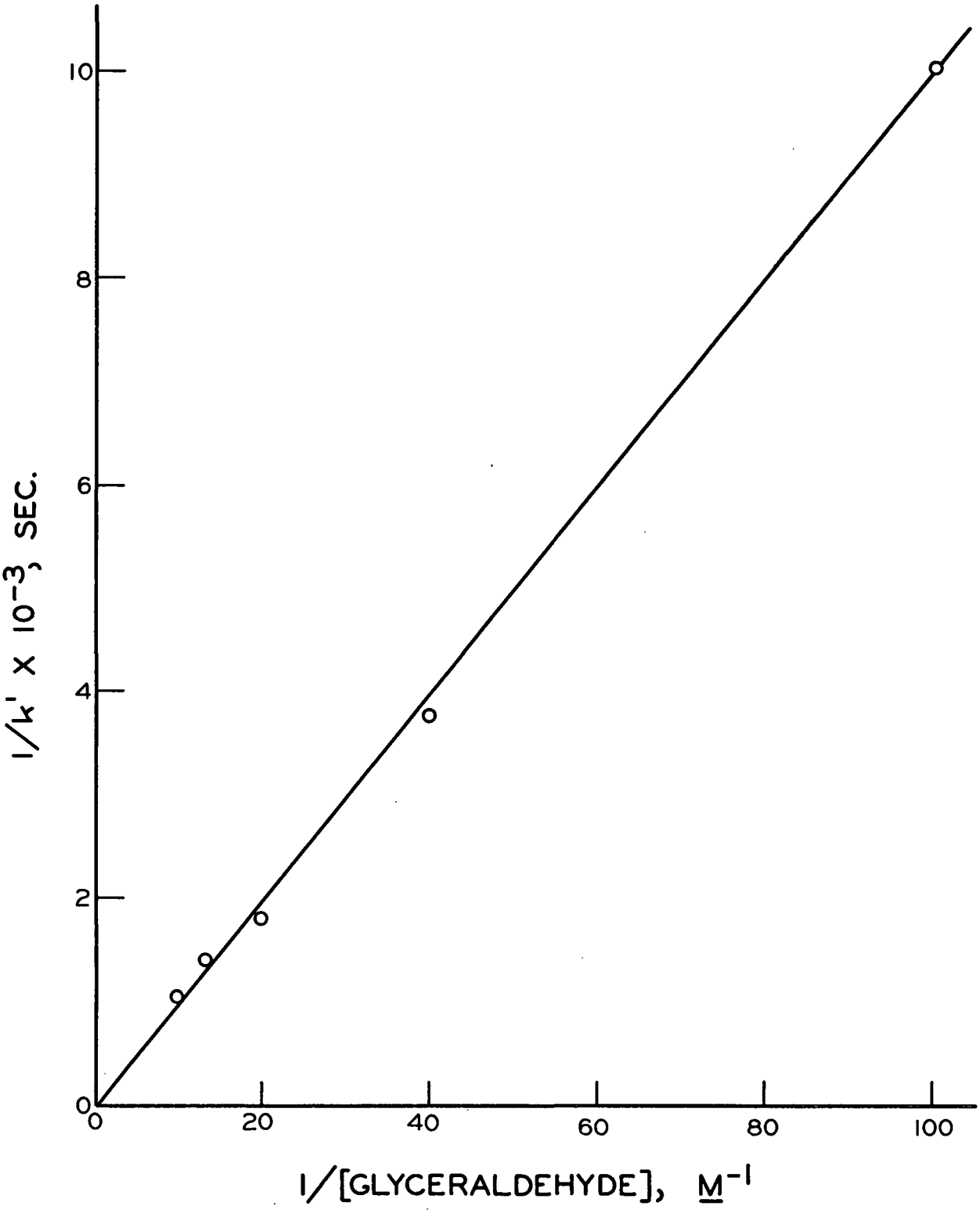


Figure 56. Duke's Reciprocal Plot for the Oxidation of Glyceraldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.100M $HClO_4$ at 70.0°C.

where $[Fe^{+3}_t]$ is now only the sum of $[Fe^{+3}_{aq}]$ and $[FeOH^{+2}]$,

$$[Fe^{+3}_t] = [Fe^{+3}_{aq}] + [FeOH^{+2}]. \quad (38)$$

From Equations (38) and (28), $[Fe^{+3}_{aq}]$ may be obtained as a function of $[Fe^{+3}_t]$,

$$[Fe^{+3}_{aq}] = [Fe^{+3}_t]/(1 + K_h/[H^+]). \quad (39)$$

From Equations (28) and (39), the rate expression given by Equation (37) may be transformed into

$$-d[Fe^{+3}_t]/dt = 2 \frac{k_1[H^+] + k_2K_h}{[H^+] + K_h} [S] [Fe^{+3}_t]. \quad (40)$$

This can be put into the form of a pseudo-first-order equation,

$$-d[Fe^{+3}_t]/dt = k' [Fe^{+3}_t] \quad (41)$$

where

$$k' = 2 \frac{k_1[H^+] + k_2K_h}{[H^+] + K_h} [S]. \quad (42)$$

Examination of Equation (42) shows that when $[H^+] >> K_h$,

$$k' \approx 2(k_1 + k_2K_h/[H^+]) [S], \quad (43)$$

which is the equation given by Thomas, Trudel and Bywater (15) for the ferric perchlorate oxidation of acetoin and predicts a linear dependence of k' upon $1/[H^+]$.

Inversion of Equation (43) gives

$$1/k' = \frac{1}{2}\left(\frac{[H^+] + K_h}{k_1[H^+] + k_2K_h}\right) \frac{1}{[S]}, \quad (44)$$

which is approximated by

$$1/k' = \frac{1}{2}\left(\frac{[H^+]}{k_2K_h} + \frac{1}{k_2}\right) \frac{1}{[S]}, \quad (45)$$

when $k_1 [H^+] \ll k_2 K_h$. The latter equation implies a linear relationship between $1/k'$ and $[H^+]$, which is the case for the oxidation of glyceraldehyde at 70°C.

From Equation (43), it can be seen that the intercept on the k' axis of a plot of k' versus $1/[H^+]$ is $2k_1[S]$. In Fig. 52, this intercept appears to be negative, but since a negative rate constant is impossible, this is taken to mean that k_1 is very small or zero. From Equation (45) and Fig. 53, k_2 is estimated to be 0.133 liter $mole^{-1}$ $sec.^{-1}$ Thus, the noncomplexing hypothesis predicts that essentially all oxidation of glyceraldehyde by ferric perchlorate at 70°C. occurs via $FeOH^{+2}$, whereas the complexing mechanism predicts the observed kinetics if it is assumed that all oxidation occurs via Fe^{+3}_{aq}.

The temperature dependence of the reaction is shown in Fig. 57 and 58. An activation energy of 37.6 kcal./mole was calculated from the slope of the Arrhenius plot (Fig. 58).

Product Analysis

A glyceraldehyde oxidation mixture whose original composition was 0.005M glyceraldehyde, 0.00972M $Fe(ClO_4)_3$, 0.050M $HClO_4$, and 0.95M $NaClO_4$ was reacted until the ferric ion concentration dropped to 2.5×10^{-4}M. Aliquots (25 ml.) of the cooled reaction mixture and of 0.005M glyceraldehyde solution were mixed with 25-ml. portions of saturated 2,4-dinitrophenylhydrazine in 2M HCl.

After 10 minutes at room temperature, the precipitates were filtered off, dried, and weighed. The reaction mixture gave 15.9 mg. of precipitate while the glyceraldehyde solution gave 0.7 mg. From the change in ferric ion concentration, a weight of 53.3 mg. is to be expected if glyceraldehyde is converted quantitatively to hydroxypyruvaldehyde. The low yield may have been due to the short reaction time allowed for derivatization or to secondary oxidation of hydroxypyruvaldehyde to

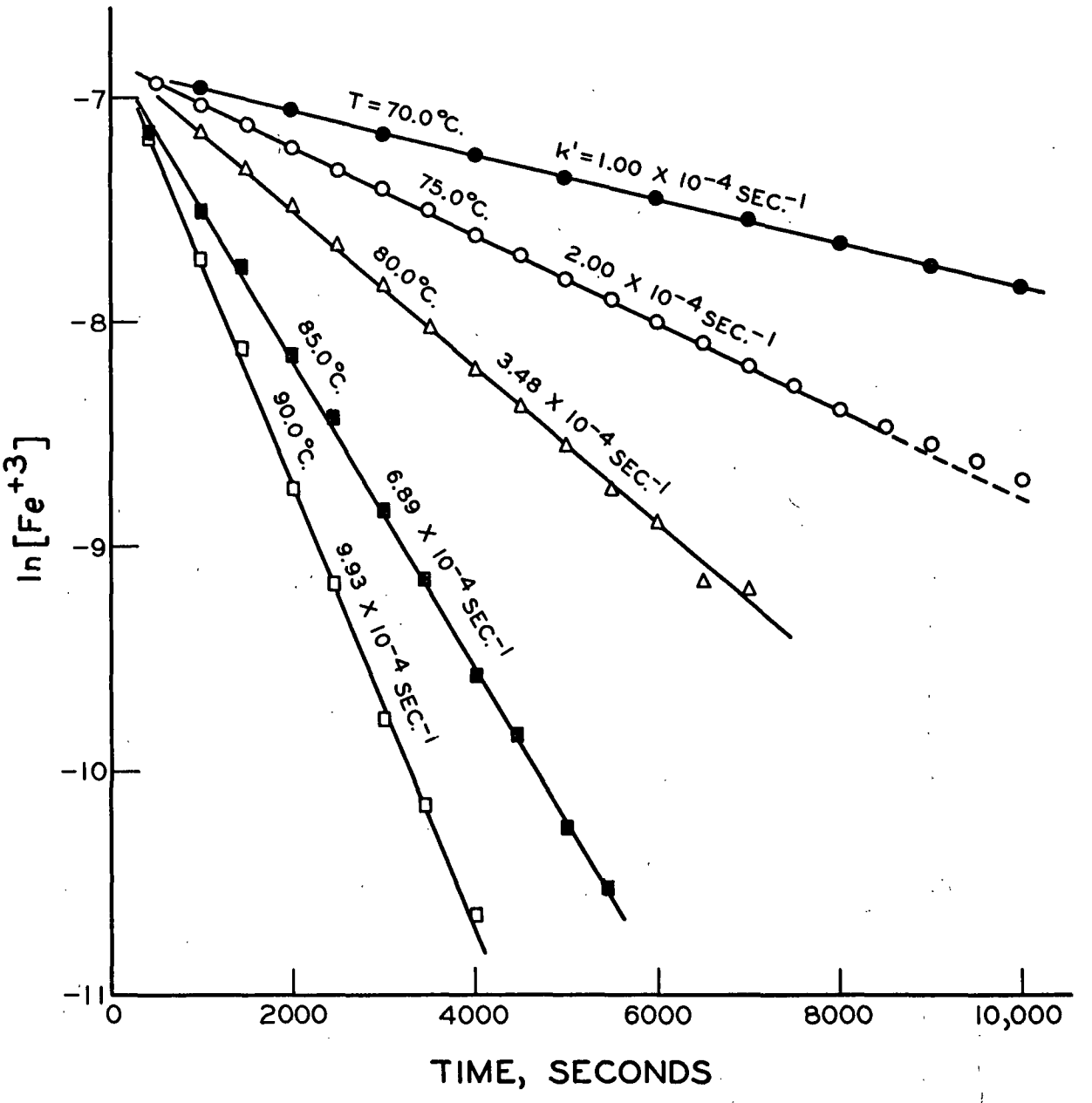


Figure 57. The Effect of Temperature on the Oxidation of 0.0100M Glyceraldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.100M $HClO_4$

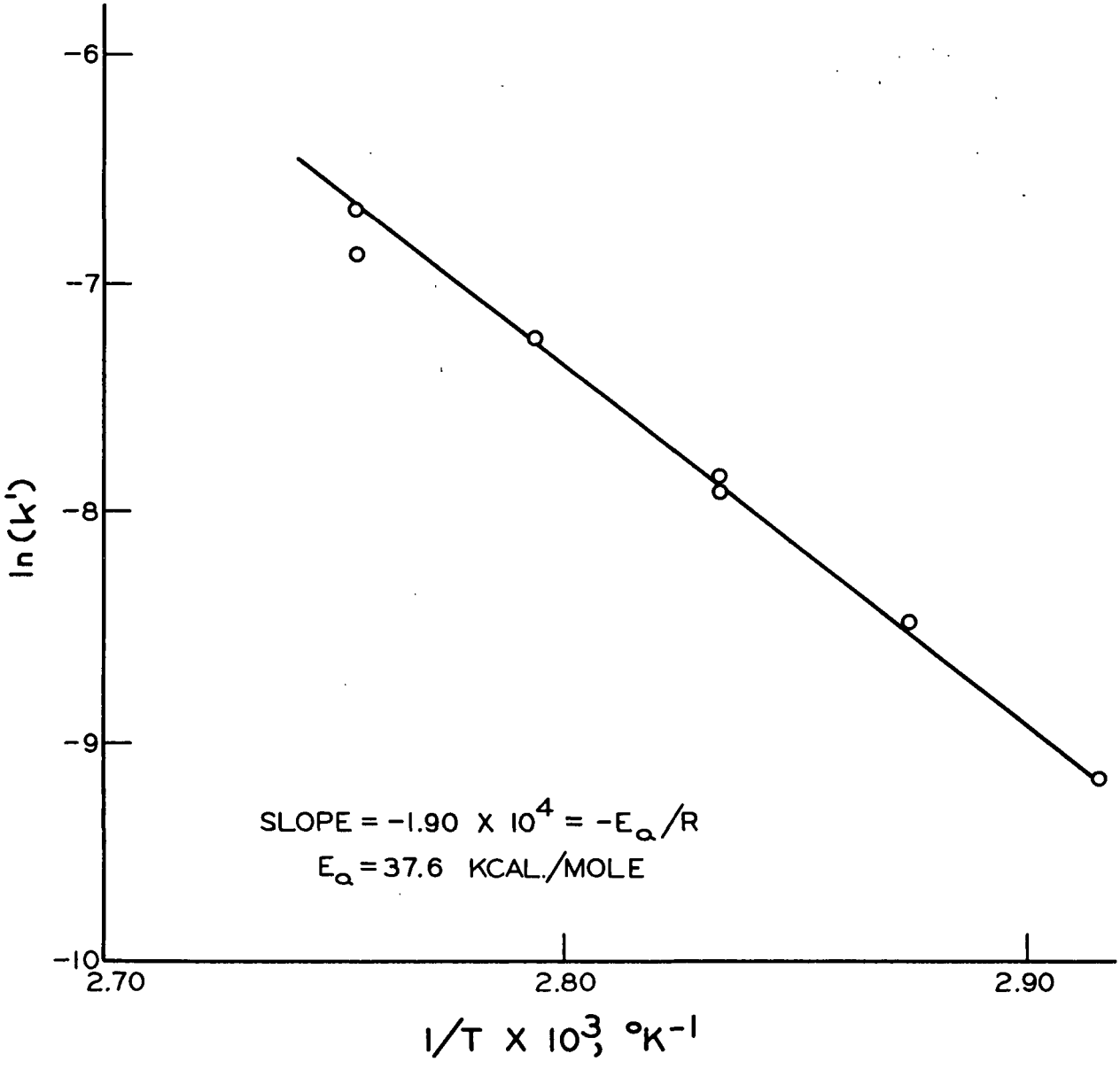


Figure 58. The Arrhenius Plot for the Oxidation of 0.0100M Glyceraldehyde by 10^{-3}M $Fe(ClO_4)_3$ in 0.100M $HClO_4$

noncarbonylic products. Secondary oxidation of some sort is likely to have occurred since the oxidation was not run with excess glyceraldehyde.

The DNPH precipitate was chromatographed on thin-layer plates in benzene-tetrahydrofuran mixtures. Three compounds were separated. The most abundant component had a chromatographic mobility similar to that of glyceraldehyde 2,4-dinitrophenylosazone. It probably arose from hydroxypyruvaldehyde (glycerosone) in the reaction mixture. The next most abundant component's mobility corresponded with that of pyruvaldehyde bis-2,4-dinitrophenylhydrazone. (Glyceraldehyde is known to undergo hydroxymethyl group reduction when derivatized with 2,4-dinitrophenylhydrazine (57). The same may be true for glycerosone.) The third component had an R_g value of 0.26 in benzene/tetrahydrofuran (80:20) and 0.08 in benzene/tetrahydrofuran (93:7). Its identity is unknown. It does not appear to be mesoxaldehyde tris-2,4-dinitrophenylhydrazone since the R_g value of this compound in benzene/tetrahydrofuran (93:7) has been reported to be 0.44 (55).

Proposed Reaction Mechanism

From the results, it seems probable that glyceraldehyde is oxidized by ferric ion by a mechanism similar to that proposed for glycolaldehyde. The fact that glyceraldehyde oxidations follow pseudo-first-order kinetics at lower concentrations of acid than does glycolaldehyde probably means that the rate of enolization of glyceraldehyde is considerably faster than that for glycolaldehyde.

Except at low acid concentrations, the oxidation of glyceraldehyde is considerably slower than the oxidation of glycolaldehyde. This may be the result of some ferric ion being tied up in an unreactive complex in the case of glyceraldehyde. The additional hydroxyl group of glyceraldehyde (as compared with glycolaldehyde) may participate in a chelate complex with the C-2 hydroxyl and ferric ion. Since complex formation between ferric ion and α-glycols does not

seem to result in oxidation-reduction (at least not at an appreciable rate), a complex involving the C-2 and C-3 hydroxyls of glyceraldehyde would probably be unreactive. The net effect of formation of this type of complex would be to reduce the concentration of the free ferric ion, hence the concentration of the reactive enediol complex also. This would lower the overall rate of oxidation of glyceraldehyde in comparison with glycolaldehyde.

Formation of similar unreactive complexes may be postulated for glucose, also. Complexes involving the C-2 — C-3, C-3 — C-4, or the C-4 — C-6 glycol pairings might compete for ferric ion with the 1,2-enediol of glucose.

CONCLUSIONS

The model compound study established that primary and secondary alcohol groups, α-glycol groupings, and the aldehyde function are all relatively unreactive with respect to the ability to reduce ferric ion. The results suggest that the cyclic hemiacetal form of sugars is not readily attacked by ferric ion. The ability of sugars to reduce ferric ion at an appreciable rate seems to be related to the fact that the cyclic hemiacetal form is in equilibrium with an acyclic α-hydroxyaldehyde species. The latter is postulated to be highly reactive toward ferric ion.

The oxidation of α-hydroxycarbonyl compounds probably proceeds via complex formation between their enediol forms and ferric ion. Glucose was found to form a 1:1 complex with ferric ion. There was some evidence that glyceraldehyde, glycolaldehyde, and trans-1,2-cyclohexanediol form 1:2 complexes (two substrate molecules) at higher substrate concentrations. Trans-1,2-cyclohexanediol did not reduce ferric ion at an appreciable rate, probably because a free radical formed by disproportionation of the complex would not be resonance-stabilized as in the case of enediol compounds.

The oxidations of glucose and glycolaldehyde were shown to initiate polymerization of methyl methacrylate, indicating that the first oxidation products are free radicals. The latter are then oxidized to α-dicarbonyl compounds. Glyoxal, the product of oxidation of glycolaldehyde, is resistant to further oxidation, but glucosone, which appears to be the initial stable product of oxidation of glucose, seems to be readily attacked by ferric ion, leading ultimately to formation of two- and three-carbon carbonyl compounds by carbon-carbon bond cleavage. Cleavage is hypothesized to be a result of oxidation of glucosone to the 2,3-diketo aldose, with the latter hydrolyzing at a carbon-carbon bond.

From what has been learned about ferric ion oxidations of glucose and related model compounds, it seems unlikely that an oxidative mechanism would be very important in acceleration of degradation of relatively pure cellulose by ferric ion. This conclusion is based upon the fact that α-hydroxycarbonyl groupings, which are preferentially oxidized by ferric ion, are relatively scarce in pure cellulosic materials. Reactive sites in cotton linters, for example, would occur only at the reducing ends of cellulose molecules and at the occasional carbonyl groups in the 2- and/or the 3-carbon positions.

In the case of bleached cellulosic materials, oxidative attack by ferric ion may be an important factor in degradation since bleaching introduces carbonyl groups into cellulose.

EXPERIMENTAL

REAGENTS

FERRIC PERCHLORATE

Ferric perchlorate was purchased from K&K Laboratories as the hexahydrate. A stock solution was prepared by dissolving the salt in triply distilled water (67) and adding perchloric acid to prevent hydrolysis. The stock solution was analyzed for ferric ion content by titration with ceric ammonium sulfate (standardized against reagent-grade iron wire) after reduction of ferric ion with stannous chloride (68).

The acid concentration was calculated by determining the total cation concentration and subtracting from this the ferric ion concentration. The total cation concentration was found by running measured aliquots of the stock solution through IR-120 (H^+) and titrating the effluent with base to determine the resultant acid concentration. This method was checked by direct titration of the stock solution with base. Direct titration also gives the total cation concentration since ferric ion consumes base stoichiometrically to form the hydrous oxide. Values of the total cation concentration obtained by the two methods were in excellent agreement with one another.

PERCHLORIC ACID

Mallinckrodt reagent-grade perchloric acid was diluted with triply-distilled water to give approximately 5M solutions. Aliquots of stock solutions were titrated with standardized sodium hydroxide solutions to phenolphthalein end points. Sodium hydroxide solutions were prepared from commercial CO_2-free concentrates and were standardized against potassium hydrogen phthalate.

Less concentrated stock solutions were made up as required by dilution of 5M solutions with appropriate quantities of triply-distilled water.

SODIUM PERCHLORATE

Sodium perchlorate was prepared by neutralizing reagent-grade perchloric acid solutions with carbonate-free sodium hydroxide solutions by the method of Aveston (69). The resulting sodium perchlorate solutions were analyzed by evaporating aliquots to dryness at 110°C. and weighing the anhydrous salt. The gravimetric method was checked by comparison with an ion exchange method of analysis. Agreement between the two methods was excellent.

CYCLOHEXANOL

Cyclohexanol which had been previously purified by Schroeder (70) was fractionally distilled on a spinning band column. Fractions which boiled at 71°C. at a pressure of 15.5 mm. Hg [lit. b.p. 56°C., 10 mm. (71)] were taken from the middle of the distillation.

ISOBUTYL ALCOHOL

Isobutyl alcohol (Matheson, Coleman, and Bell) was fractionally distilled on a spinning band column. The fraction taken for kinetic study had a boiling point at atmospheric pressure of 108°C. [lit. b.p. 108.4°C. (72)].

2-BUTANOL

2-Butanol (Matheson, Coleman, and Bell) was fractionally distilled at atmospheric pressure on a spinning band column, b.p. 99°C. [lit. b.p. 99.5-100°C. (73)].

TRANS-1,2-CYCLOHEXANEDIOL

trans-1,2-Cyclohexanediol (Aldrich Chemical Co.) was recrystallized from benzene three times, m.p. 103.5-104.5°C. [lit. m.p. 101.5-103.0°C. (74)].

CIS-1,2-CYCLOHEXANEDIOL

cis-1,2-Cyclohexanediol [prepared by Hintz (75)], m.p. 99.4-100°C. [lit. m.p. 98°C. (76)] was used without further purification.

1,5-ANHYDROGLUCITOL

1,5-Anhydroglucitol [prepared by Pottenger (20)] was recrystallized from ethanol, m.p. 141-142°C. [lit. m.p. 142-143°C. (77)].

BUTYRALDEHYDE

Butyraldehyde was fractionally distilled on a spinning band column. A fraction boiling at 74°C. [lit. b.p. 74°C. (63)] at atmospheric pressure was taken for kinetic experiments.

GLYCOLALDEHYDE

Glycolaldehyde (Aldrich Chemical Co.), m.p. 81-83.5°C. [lit. m.p. 81-82°C. (78)] was used as received since it proved to be very difficult to recrystallize. Its NMR and IR spectra indicated a high degree of purity.

GLYCERALDEHYDE

D,L-Glyceraldehyde (K&K Laboratories), m.p. 139-140.5°C. [lit. m.p. 137-139°C. (79)] was used without purification.

GLUCOSE

Baker reagent-grade anhydrous dextrose was used without purification. Paper chromatograms of this sugar in several solvents showed only one spot after development with ammoniacal silver nitrate.

3-O-METHYLGLUCOSE

3-O-Methylglucose (Pierce Chemical Co.), m.p. 163.5-164.5°C. [lit. m.p. 163-164°C. (80)] was used as received. Paper chromatography showed that the sample was glucose-free within the limits of detection.

2,3,4,6-TETRA-O-METHYLGLUCOSE

2,3,4,6-Tetra-O-methylglucose [prepared by Hintz (75)], was analyzed by Pottenger (20). Gas chromatography gave only one peak. The melting point was 85-86°C. (20), compared with literature values of 83-85°C. (81) and 89-90°C. (82).

2-DEOXYGLUCOSE

2-Deoxy-D-glucose (Calbiochem) had a melting point of 141.5-144.5°C. as received [lit. m.p., 142-144°C. (83)]. It was used without purification.

TECHNIQUES

KINETICS

Manual Sampling Method

Some oxidations of glucose were carried out in sealed glass ampoules. The procedure used for these reactions was as follows. Reaction mixtures were made up, and measured aliquots were pipetted into 5-ml. glass ampoules. The ampoules and their contents were purged with prepurified nitrogen. After an hour of purging,

the purging tubes were withdrawn from the ampoules, which were immediately stoppered with modeling clay. The ampoules were sealed by heating their necks in a small flame, puncturing the clay stoppers to prevent pressure build-up and drawing out the necks when the glass became soft.

The sealed ampoules were then placed in a controlled temperature (± 0.1°C.) bath and the time noted. Ampoules were withdrawn from the bath at varying time intervals, opened, and their contents were analyzed for ferrous ion by a modified o-phenanthroline colorimetric method (see Appendix I).

Automatic Sampling Method

Most kinetic studies were conducted with an automatic sampling and analysis method. In this method, one of the reactants (either the ferric ion or the substrate solution) is placed in a thin-walled glass-ampoule fused to a glass rod, and the remaining components of the reaction mixture are put into a 100-milliliter round-bottomed, one-necked flask. The glass rod attached to the ampoule is put through one hole in a 3-holed rubber stopper. The rubber stopper is then seated in the mouth of the round-bottomed flask. (See Fig. 59.)

Nitrogen inlet and outlet tubes are placed through the remaining holes in the rubber stopper. The nitrogen-inlet tube is connected to a tank of prepurified nitrogen. Nitrogen is bubbled through the reaction mixture (stopcock on the outlet tube open) for about an hour. Then the stopcock is closed, a clamped-off sample withdrawal line is attached to the nitrogen-inlet tube, and a nitrogen-filled balloon is attached to the nitrogen-outlet tube. The flask is placed in a constant-temperature oil bath after opening the stopcock on the nitrogen-outlet tube.

The flask is kept in the oil bath for at least one hour (for temperature equilibration) before initiating the reaction. Just prior to initiation, the

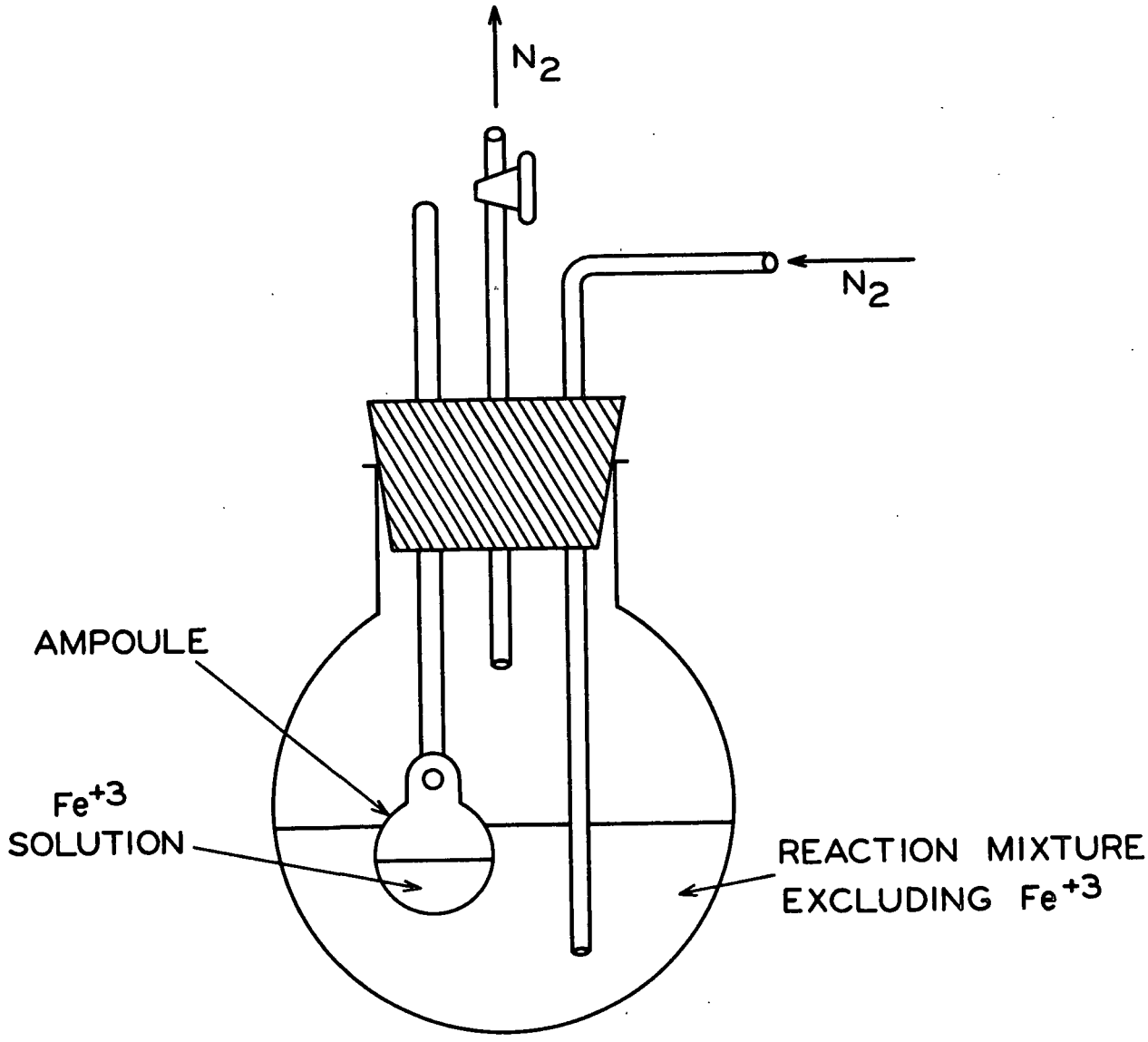


Figure 59. The Reaction Flask for the Automatic Sampling and Analysis for Following Reactions

sample withdrawal line is connected to a Technicon Auto Analyzer proportioning pump, and the line is unclamped. The function of the pump is to draw reaction mixture from the reaction flask, mix it with colorimetric reagents, and pump it through a flow cell placed in a Cary Model 15 recording spectrophotometer. (See Appendix II.)

Initiation is accomplished by pushing down on the glass rod, breaking the ampoule on the bottom of the reaction flask. At the same time, the proportioning pump and the recorder on the spectrophotometer are turned on. Mixing is accomplished by swirling the flask vigorously by hand for a few seconds. The reaction is followed by recording the change in the absorbance of the complexed reaction mixture as a function of time.

PRODUCT ANALYSIS

Some attempts were made to isolate products of glucose oxidations by paper chromatography and gas chromatography of trimethylsilyl derivatives. These results were unsuccessful, however, owing to the small concentrations of products and large concentrations of unreacted glucose in reaction mixtures. No satisfactory method could be found for separating the products themselves from glucose. The problem was solved by treating reaction mixtures with 2,4-dinitrophenylhydrazine under conditions such that only the products and not the unreacted glucose were derivatized. This procedure was made possible by the fact that at least some of the reaction products were carbonyl compounds.

Derivatization with 2,4-Dinitrophenylhydrazine.

Derivatization was carried out according to the procedure of Iddles and Jackson (84). Saturated solutions of 2,4-dinitrophenylhydrazine in 2M hydrochloric acid were added to equal volumes of the solutions to be derivatized.

After standing for the desired times (generally at room temperature) precipitates were filtered out of the mixtures and dried in a vacuum desiccator over calcium chloride.

Unreacted glucose did not interfere when glucose reaction mixtures were treated with 2,4-dinitrophenylhydrazine, since glucose solutions were found to give negligible amounts of precipitate after six hours standing with 2,4-dinitrophenylhydrazine at room temperature.

Chromatography of 2,4-Dinitrophenylhydrazine Derivatives

The mixtures of benzene and tetrahydrofuran used by Byrne (55) were found to be the most useful solvents for thin-layer chromatography of 2,4-dinitrophenylhydrazine derivatives. Qualitative analyses were carried out on microscope slides coated with silica gel as reported by Peifer (53). Quantitative thin-layer work utilized 10 cm. x 20 cm. glass plates coated with silica gel by a Camag spreader. Some preparative work was carried out by column chromatography on Mallinckrodt SilicAR, 100-200 mesh.

Identification of 2,4-Dinitrophenylhydrazine Derivatives

Generally, identifications were made only on the basis of chromatographic comparison with known compounds. Since this is insufficient evidence for identification of a compound, such identifications must be considered to be tentative.

In the case of the major 2,4-dinitrophenylhydrazine derivatives obtained from glucose oxidation mixtures, however, additional evidence was obtained by comparison of their x-ray diffraction patterns with those of known compounds. After separation by column chromatography and purification by preparative thin-layer chromatography, 2,4-dinitrophenylhydrazine derivatives were packed into

thin-walled glass capillaries and exposed to an x-ray beam in a Debye-Scherrer camera for six hours. The x-ray diffraction patterns obtained from known and unknown compounds were compared.

COMPLEXING

Spectrophotometric studies were used to show the existence of complexes between ferric ion and the substrates glucose, trans-1,2-cyclohexanediol, glycolaldehyde and glyceraldehyde.

Stock solutions of ferric perchlorate in perchloric acid and stock solutions of the substrates were prepared with triply-distilled water. Measured amounts of either the ferric ion or the substrate solutions were put into a spectrophotometer cell, water was added, if necessary, and the remaining component was added by means of a syringe fitted with a Chaney adapter. The cell was placed in a Cary Model 15 recording spectrophotometer and the absorbance was read at the chosen wavelength within 30 seconds of the time of mixing. The spectrophotometer cell and the syringe were then washed and dried, and the process was repeated for a different concentration of the component under study.

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APPENDIX I

THE o-PHENANTHROLINE METHOD FOR DETERMINATION OF FERROUS ION

The standard o-phenanthroline method (85) for determination of ferrous ion could not be used without modification to analyze reaction mixtures. One modification was the addition of potassium ion to reaction mixtures. This was done in order to precipitate out perchlorate as its slightly soluble potassium salt. The presence of perchlorate interferes in the o-phenanthroline method by causing o-phenanthroline to precipitate out as its perchlorate salt (86).

A second modification of the standard method was addition of fluoride ion. This was done to tie up residual ferric ion in the stable fluoride complex. The purpose was to prevent reaction between residual ferric ion and unreacted substrate in the complexing medium. Without the presence of fluoride ion, reduction of ferric ion was relatively rapid in the complexing medium at room temperature. [Ferriin, tris-1,10-phenanthrolineiron (III), has been shown to oxidize cyclohexanone and other organic compounds rapidly at relatively low temperatures (5-25°C.) in acidic media (42).] Fluoride ion greatly decreased the rate of reduction of ferric ion in the presence of o-phenanthroline, as shown by Fig. 60.

The procedure for the modified o-phenanthroline is as follows. Four milliliters of 4M KCl and one milliliter of 0.657M KF are pipetted into a 25-milliliter volumetric flask. A two-milliliter sample of reaction mixture is transferred to the flask and the contents are swirled. One milliliter of 0.5% o-phenanthroline solution is added, followed by two milliliters of 8M potassium acetate and four milliliters of 0.657M KF. After dilution to volume, the mixture is cooled for about ten minutes in running water at about 10°C. The solution is filtered through Whatman No. 50 filter paper to remove precipitated potassium

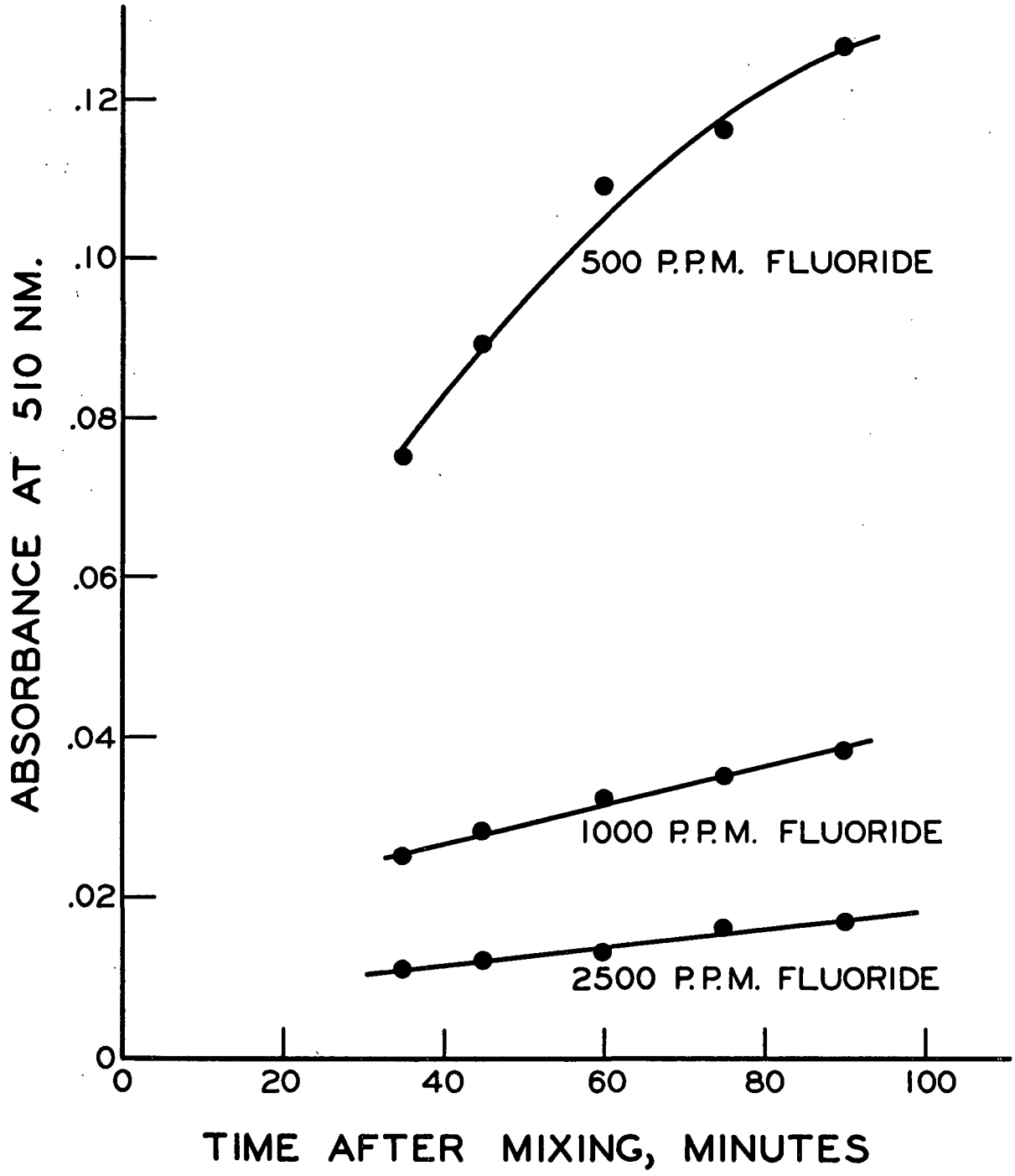

Figure 60. The Effect of Fluoride on Rate of Ferrous Ion Production in Complexed Glucose Reaction Mixtures*

*Five milliliters of a reaction mixture containing 1.00N $HClO_4$, 0.001N $Fe(ClO_4)_3$ and 0.10M glucose were complexed with O-phenanthroline and diluted to 25 ml.

perchlorate. The absorbance of the filtrate is read at 510 nm. on a Beckman DU spectrophotometer approximately thirty minutes after the addition of potassium acetate. The calibration curve for this method is shown in Fig. 61.

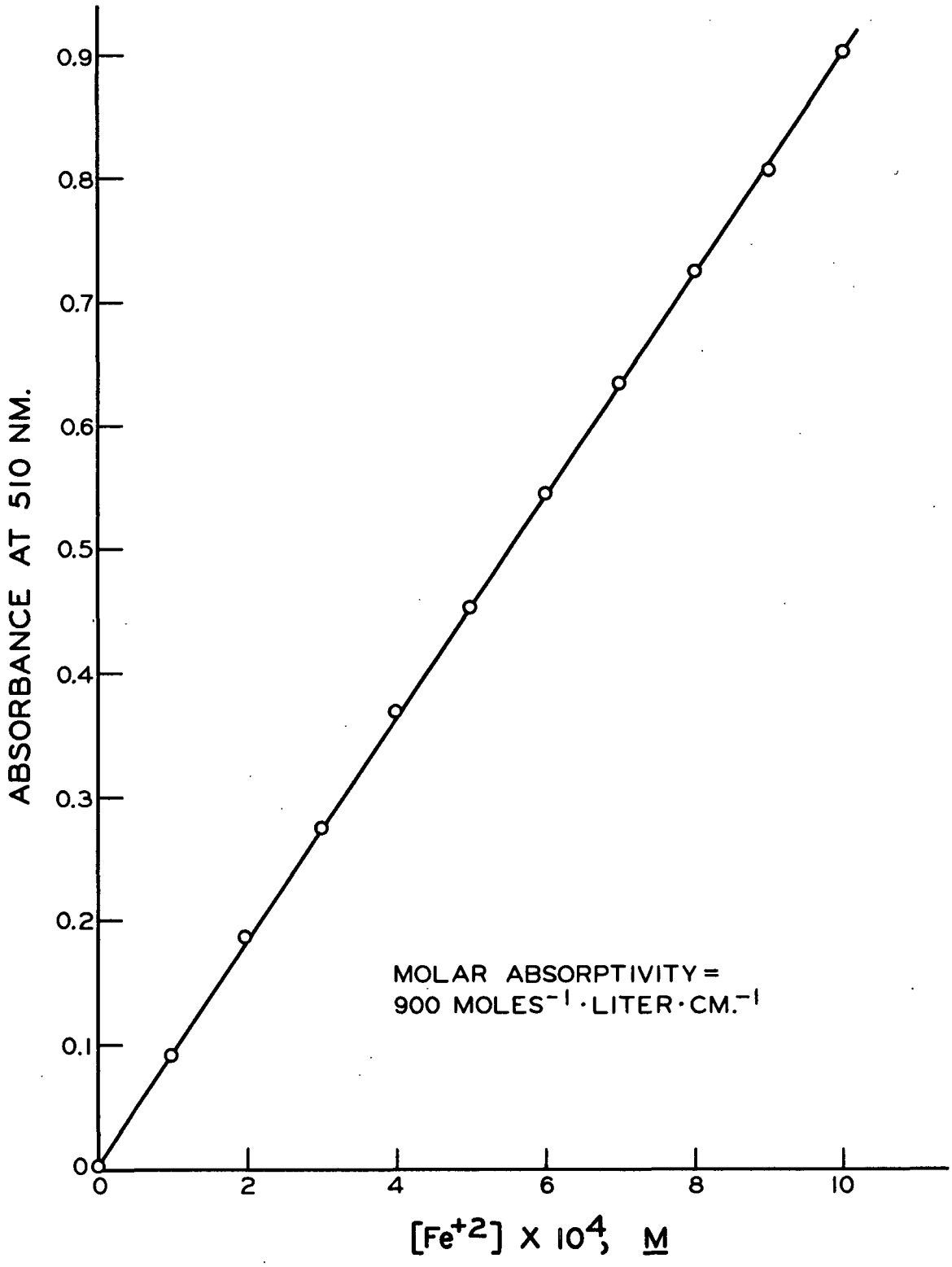

Figure 61. Calibration Curve for the o-Phenanthroline Method of Ferrous Ion Determination

APPENDIX II

THE AUTOMATIC METHOD OF FERRIC ION DETERMINATION

The automatic method of analysis for ferric ion was described briefly in the section, Automatic Sampling Method (p. 139). Figure 62 gives a more detailed picture of the analysis procedure. The AutoAnalyzer proportioning pump draws reaction mixture from the reaction flask and adds to it 0.10M HCl and nitrogen bubbles (for mixing purposes). The solution then goes through a mixing coil, after which 0.73M NH_4SCN is added to it. Another mixing coil and a tee, which removes the nitrogen bubbles, precede a Beckman Micro-Aperture flow cell placed in a Cary Model 15 recording spectrophotometer. The spectrophotometer continuously measures and records the absorbance of the solution passing through the flow cell.

Although the ferric thiocyanate complex supposedly does not follow Beer's law (87), Fig. 63 shows no deviation from linearity over the range of concentrations of ferric ion which was studied.

The absorbance of a standard ferric ion solution complexed by the AutoAnalyzer method was found to vary somewhat from day to day. This was believed to be caused by changes in diameters of tubing in the proportioning pump as a result of constant stretching and relaxation during operation of the pump. To counteract this, two-point calibrations were carried out just prior to each run. The spectrophotometer was adjusted to read zero when distilled water was put through the reaction mixture line, giving one calibration point. The second point was obtained by running a standard ferric ion solution of known concentration through the reaction mixture line. From the absorbance of the complexed standard ferric ion solution, an absorptivity value was calculated. This was then used to convert absorbances to ferric ion concentrations in the kinetic run which followed.

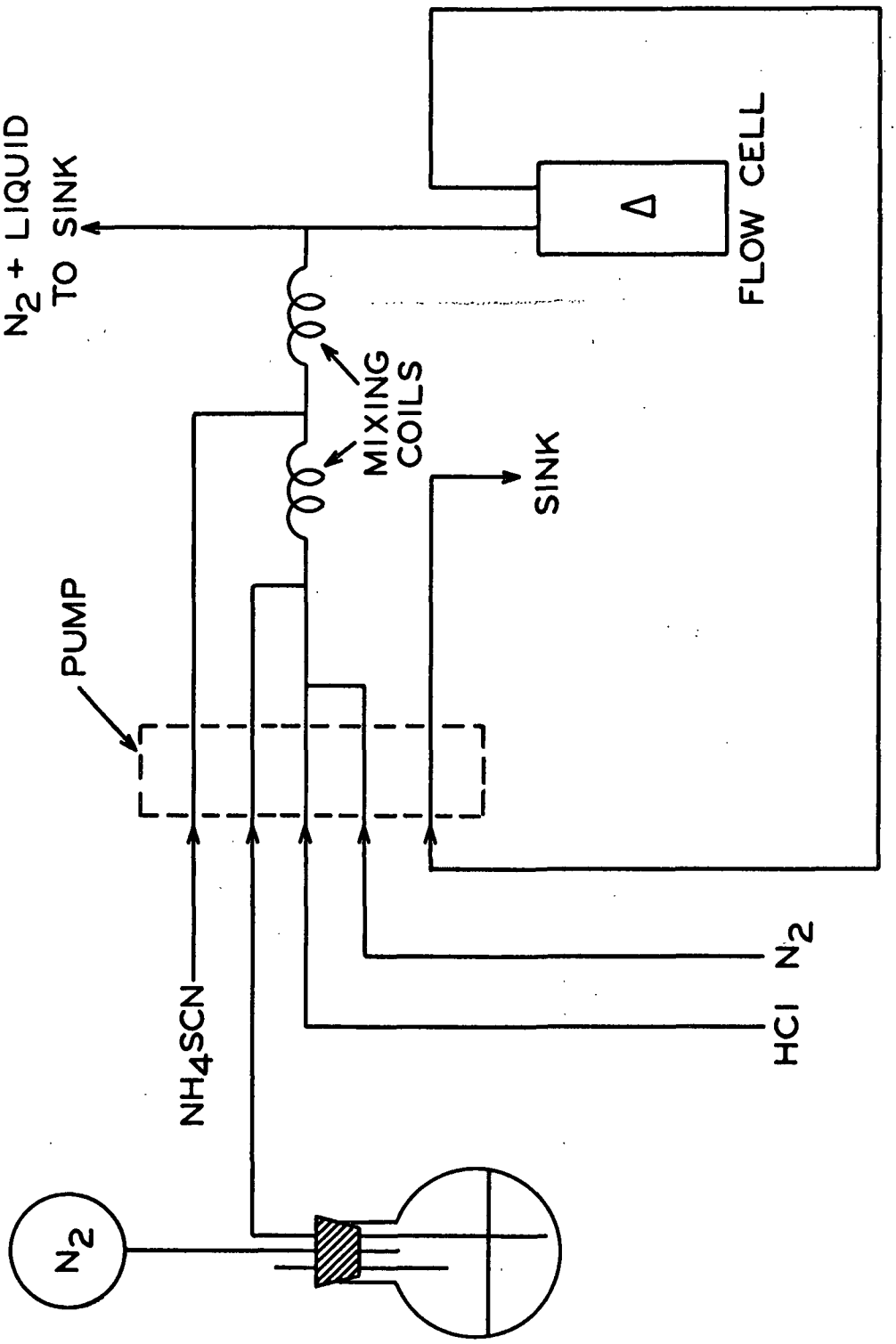

Figure 62. Schematic of the Automatic Sampling and Analysis System Used for Following the Kinetics of Oxidation-Reduction Reactions

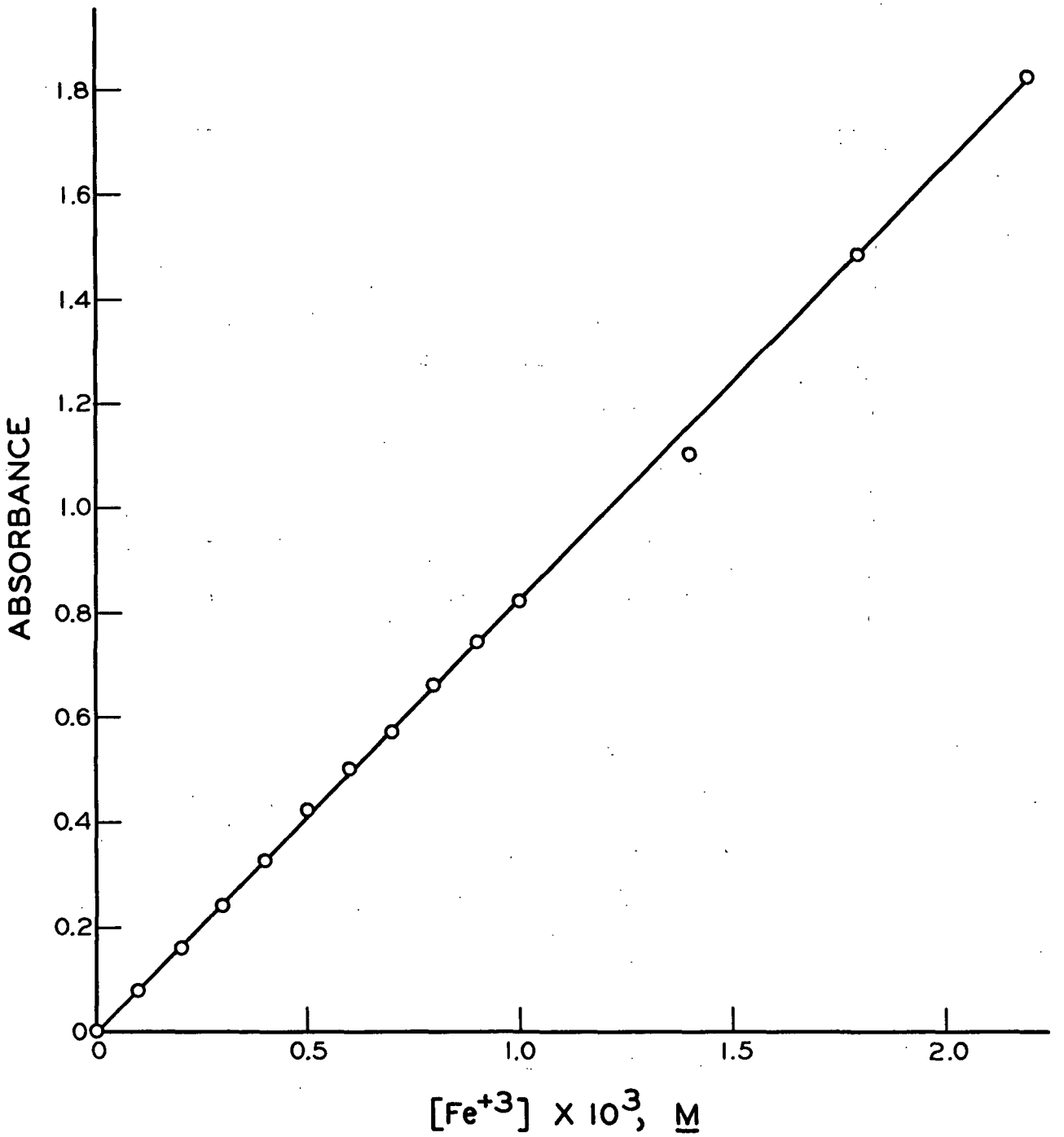

Figure 63. Calibration Curve for the Thiocyanate Method of Ferric Ion Determination

APPENDIX III

REPRODUCIBILITY OF RATE CONSTANTS

MANUAL SAMPLING AND ANALYSIS METHOD

Eight replications of the reaction between 0.10M glucose and 10^{-3}M ferric perchlorate in 1.00M $HClO_4$ were made at 88.6°C. by the manual sampling and analysis method. The rate constants which were obtained are given in Table V along with their standard deviation and coefficient of variation (Reaction 1).

Rate constants were generally used only for determining the effects of variables upon reaction rate. For this purpose determinations of rate constants need not be particularly precise, and a coefficient of variation of about 10% is probably satisfactory.

AUTOMATIC SAMPLING AND ANALYSIS METHOD

OXIDATION OF GLUCOSE IN 1M $HClO_4$

Also shown in Table V are the results of replications of two oxidations of glucose in 1M $HClO_4$ at 88.6°C. followed by the automatic sampling and analysis method. Reaction 2 was carried out under the same conditions used in Reaction 1. The agreement between rate constants determined by the two methods is excellent. The average value of the rate constant for Reaction 3, 8.55×10^{-8} moles $liter^{-1}$ $sec.^{-1}$, compares with a single determination by the manual method, 8.28×10^{-8} moles $liter^{-1}$ $sec.^{-1}$. The precision of the automatic method may be somewhat less than for the manual method, but is still satisfactory.

TABLE V

REPRODUCIBILITY OF RATE CONSTANTS IN OXIDATIONS OF GLUCOSE

	Reaction 1[a,e] $k^1 \times 10^8$	Reaction 2[b,e] $k^1 \times 10^8$	Reaction 3[c,e] $k^1 \times 10^8$	Reaction 3[c,e] $k^1 \times 10^4$
	3.61	3.44	8.33	2.36
	3.61	3.86	8.53	2.44
	3.28	3.92	8.44	2.40
	3.89	3.56	9.47	2.33
	3.75	3.67	7.97	2.35
	3.83	4.14	--	--
	3.94	--	--	--
	3.72	--	--	--
Average k'	3.70×10^{-8}	3.76×10^{-8}	8.55×10^{-8}	2.38×10^{-4}
Standard deviation	0.21×10^{-8}	0.26×10^{-8}	0.56×10^{-8}	0.044×10^{-4}
Coefficient of variation	5.6%	6.8%	6.5%	1.8%

[a] Reaction conditions: 0.10M glucose, 10^{-3}M $Fe(ClO_4)_3$, 1M $HClO_4$, and 88.6°C. followed by manual sampling and analysis method.
[b] Reaction conditions: same as above — followed by automatic sampling and analysis method.
[c] Reaction conditions: 0.25M glucose, 10^{-3}M $Fe(ClO_4)_3$, 1M $HClO_4$, and 88.6°C. — followed by automatic sampling and analysis method.
[d] Reaction conditions: 0.20M glucose, 10^{-3}M $Fe(ClO_4)_3$, 0.0112M $HClO_4$, 0.99M $NaClO_4$, and 88.6°C. — followed by automatic sampling and analysis method.
[e] Units of rate constants are mole liter^{-1} sec.$^{-1}$.
[f] Units of rate constants are sec.$^{-1}$.

OXIDATION OF GLUCOSE IN 0.01M $HClO_4$

Replications were made of an oxidation of glucose in 0.01M $HClO_4$ in order to determine whether rate constants were satisfactorily reproducible in low acidity media. The results, shown in Table V under the heading Reaction 4, indicate that rate constants were highly reproducible.

APPENDIX IV

THE EFFECT OF OXYGEN

All reaction mixtures were purged with a stream of nitrogen for at least one hour to remove oxygen. It was feared that oxygen might affect the reaction rate by reacting with free radicals formed by reaction of ferric ion with substrates or by reacting with ferrous ion formed from reduction of ferric ion.

HIGH ACIDITY MEDIA

Figure 64 compares oxidations of two identical reaction mixtures [0.25M glucose, 1.00M $HClO_4$, and 10^{-3}M $Fe(ClO_4)_3$] at 88.6°C., one purged, the other not purged with nitrogen. The unpurged reaction mixture shows a lengthy initial, nonlinear phase with a continually increasing reaction rate. A constant rate is finally reached, but it is considerably slower than that of the purged reaction mixture.

The nonlinear portion of the reaction curve is probably due to reoxidation by oxygen of ferrous ion formed by reaction of ferric ion with glucose. As oxygen is consumed, its concentration decreases, accounting for the increasing rate during the initial phase of reaction. After all oxygen is consumed, the reaction returns to its normal zero-order kinetics, except that the rate is less than normal. The latter may be due to consumption of a significant amount of glucose during the time when oxygen was present, although the glucose concentration would have to have been reduced from 0.25M to 0.10M to account for the final rate constant. At any rate, the effect of oxygen in 1M $HClO_4$, is to slow the apparent rate of reduction of ferric ion.

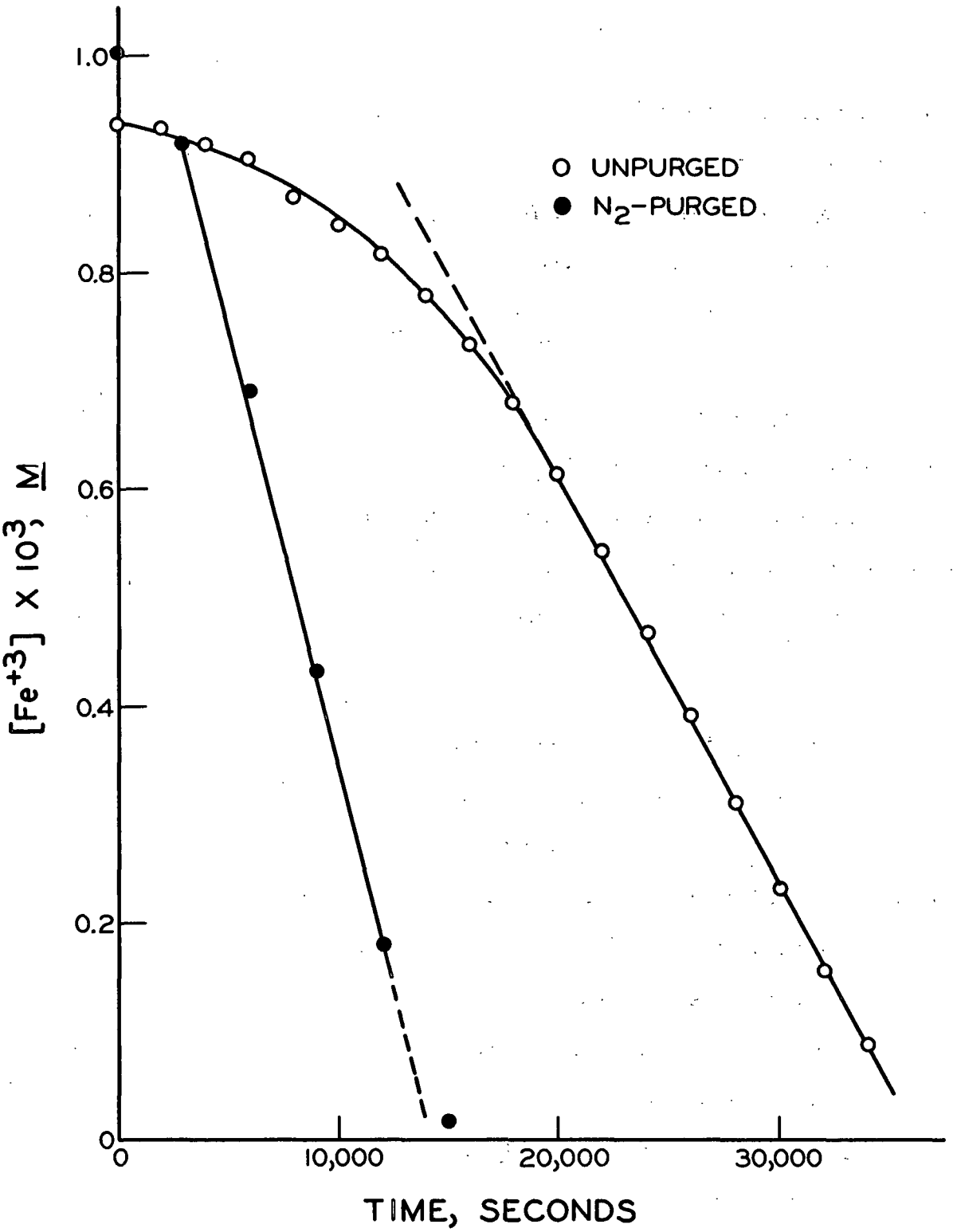

Figure 64. The Effect of Oxygen on the Oxidation of 0.250M Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 1.00M $HClO_4$ at 88.6°C.

LOW ACIDITY MEDIA

Figure 65 compares two oxidations of glucose (0.20M) in 0.01M $HClO_4$- 0.99M $NaClO_4$ by 10^{-3}M $Fe(ClO_4)_3$ at 88.6°C. One reaction was purged with nitrogen for one hour, whereas pure oxygen was bubbled through the other for 5 minutes. The results show that oxygen had very little, if any, effect on reaction rate in 0.01M $HClO_4$.

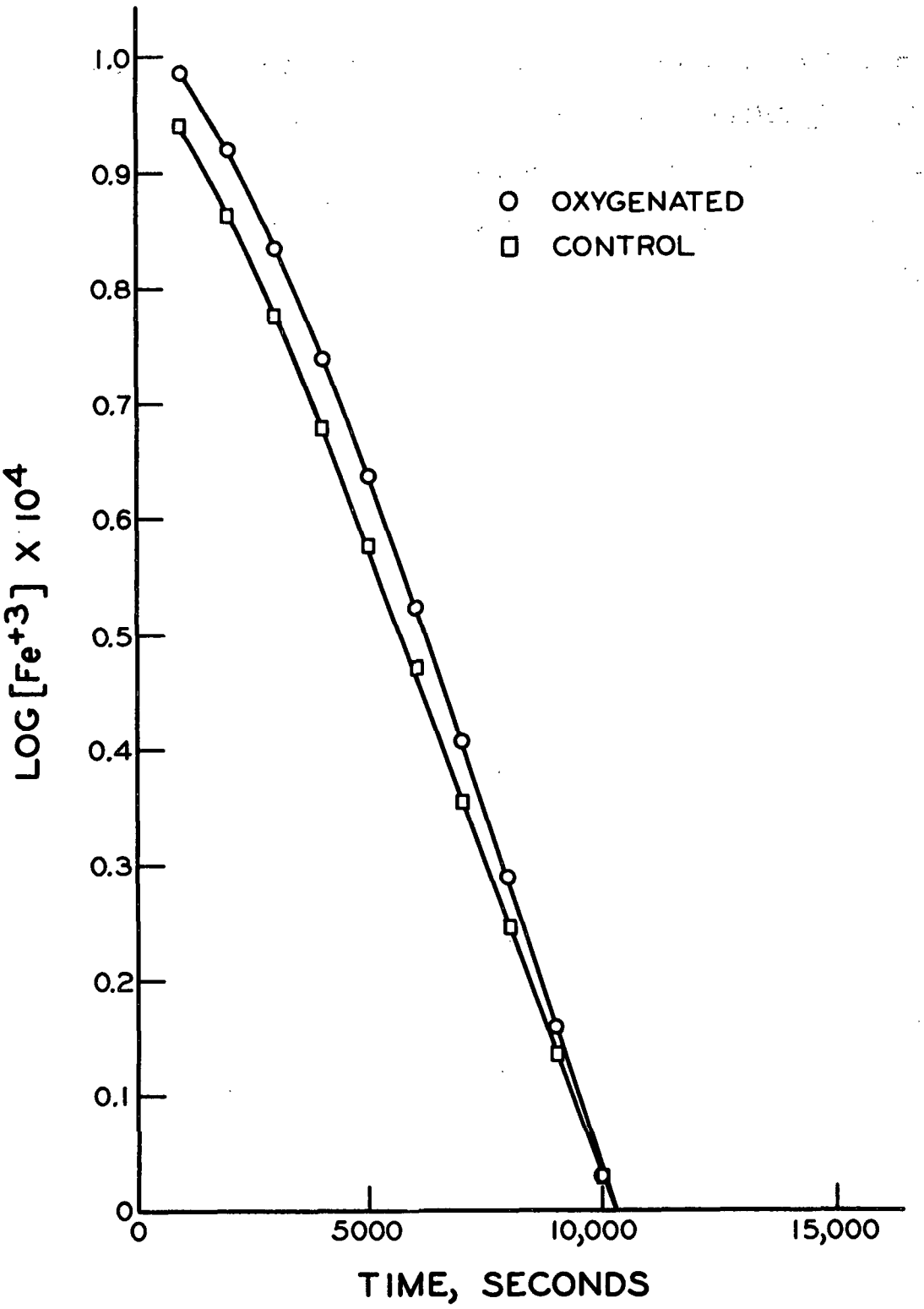

Figure 65. The Effect of Oxygen on the Oxidation of 0.200M Glucose by 10^{-3}M $Fe(ClO_4)_3$ in 0.0100M $HClO_4$ at 88.6°C.

APPENDIX V

DETERMINATION OF GLYOXAL WITH 2-AMINOBENZENETHIOL

The 2-aminobenzenethiol method of Sawicki, Hauser, and Wilson (65) was used to determine glyoxal in glycolaldehyde reaction mixtures. Reaction conditions were modified somewhat from those used previously. Instead of placing solutions in a boiling water bath for two minutes, they were put into a 60°C. bath until their absorbances ceased to increase. This modification gave solutions with a much deeper and more stable color. However, results were not very reproducible, variation seeming to be related to the age of the 2-aminobenzenethiol solution. Two calibration curves obtained by using two different 2-aminobenzenethiol solutions are shown in Fig. 66.

The results indicate that absorbance is a linear function of the glyoxal concentration. The following technique was used to determine glyoxal concentrations in glycolaldehyde reaction mixtures. Duplicate samples of 0.0003M and 0.0005M glyoxal di (sodium bisulfite) solutions were treated with 2-aminobenzenethiol at the same time as duplicate samples of reaction mixtures. The concentrations of glyoxal in glycolaldehyde mixtures were calculated by linear interpolation between average absorbances of the two known solutions.

Table VI gives the results for two oxidations of 0.01M glycolaldehyde by 0.01M ferric perchlorate, one in 1.00M $HClO_4$ at 60°C., the other in 0.1M $HClO_4$ at 70°C. Ferric ion was removed from the reaction mixtures prior to analysis by running them through IR-120(H^+). They were then diluted to 10 times their original volume.

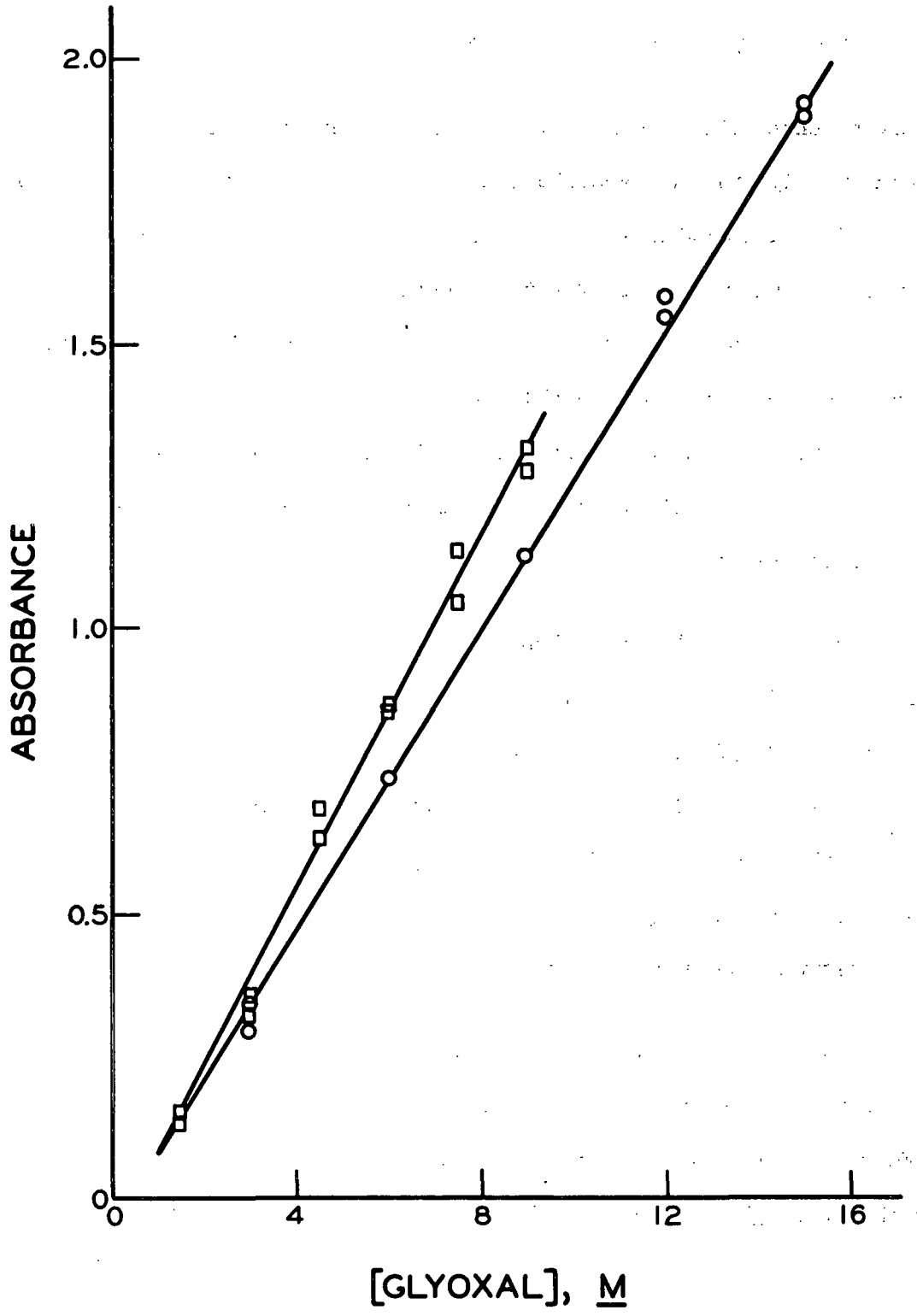

Figure 66. Two Determinations of the Relationship Between Absorbance and Glyoxal Concentration in the 2-Aminobenzenethiol Procedure (66).

TABLE VI

RESULTS OF QUANTITATIVE DETERMINATIONS OF GLYOXAL IN GLYCOLALDEHYDE REACTION MIXTURES

Determination	Absorbance (at 600 nm.) Given by 0.0003M Glyoxal	0.0005M Glyoxal	Reaction Mixture 221	Reaction Mixture 228	[Glyoxal] in Reaction Mixture, calculated
1	0.370	0.651	0.650	--	0.000479M
	0.364	0.696	0.633	--	
2	0.268	0.597	0.622	--	0.000481M
	0.309	0.658	0.569	--	
3	0.349	0.689	--	0.576	0.000412M
	0.377	0.673	--	0.496	
4	0.342	0.683	--	0.588	0.000431M
	0.380	0.684	--	0.609	

The average value of the glyoxal concentration in the sample from the 1M $HClO_4$ reaction mixture was found to be 0.000480M. Correcting for dilution, the concentration of glyoxal in the original reaction mixture was 0.00480M. The change in concentration of ferric ion during the run was from 0.01002M to 0.00075M, or 0.00927M. Assuming that 2 moles of ferric ion are required to produce 1 mole of glyoxal, the amount of glyoxal which could be formed from the amount of ferric ion which was reduced is 0.00464M. Therefore, the observed yield is 0.00480/0.00464 x 100% = 103% of the theoretical yield.

The average value found for the concentration of glyoxal in the 0.1M $HClO_4$ reaction mixture was 0.00422M, whereas the change in ferric ion concentration was 0.00972M (0.01035M-0.00063M). The yield is therefore 0.00422/0.00486 x 100% = 87% of theoretical.

APPENDIX VI

INVESTIGATION OF COMPLEXES BY THE LIMITING LOGARITHMS METHOD

The method of limiting logarithms (47) was used by Kraske (11) to determine the number of glucose molecules in the ferric ion-glucose complex. For the system,

$$mM^{+} + nL \underset{}{\overset{K}{\rightleftharpoons}} M_m L_n^{+},$$

in which

M^{+} = the metal ion,

L = the ligand molecule,

$M_m L_n^{+}$ = the complex ion,

m,n = the stoichiometric coefficients of M^{+} and L, respectively, and

K = the equilibrium constant of the coordination reaction, the following relationship can be derived from the equilibrium equation and Beer's law:

$\log A \propto m \log [M^{+}] + \log K + n \log [L]$ where

A = the absorbance of the complex,

$[M^{+}]$ = the equilibrium concentration of the "free" metal ion, and

$[L]$ = the equilibrium concentration of the "free" ligand.

If the complex concentration is small compared with the equilibrium concentrations of the metal ion and ligand molecule, $[M^{+}] \approx [M_T^{+}]$ and $[L] \approx [L_T]$, where the subscript, T, denotes the total of the "free" and combined (in the complex) species. Then, if $[M_T^{+}]$ is held constant while $[L_T]$ is varied,

$$\log A \propto n \log [L_T] + C_1,$$

where C_1 is a constant. The limiting logarithms method is based upon this relationship, for a plot of $\log A$ versus $\log [L_T]$ should yield a straight line whose slope is n, the number of molecules of ligand involved in the complex.

Kraske (11) found that the value of n for the ferric ion-glucose complex is unity, indicating that one molecule of glucose is complexed with one or more ferric ions.

In the present work, the method of limiting logarithms was used to determine the number of substrate molecules complexing with ferric perchlorate for glucose, glycolaldehyde, glyceraldehyde, and trans-1,2-cyclohexanediol. In agreement with Kraske's findings with ferric sulfate (11), Fig. 67 shows that one molecule of glucose is involved in the complex with ferric perchlorate. For trans-1,2-cyclohexanediol, glycolaldehyde, and glyceraldehyde, slopes of the limiting logarithms plots are all somewhat higher than, but reasonably close to, unity (see Fig. 68).

NUMBER OF FERRIC IONS

The method of limiting logarithms can also be used to determine the number of metal ions in a complex since, if $[L_T]$ is held constant and $[M_T^+]$ is varied,

$$\log A \propto m \log [M_T^+] + C_2.$$

The value of m may be determined from the slope of a plot of log A against log $[M_T^+]$.

Kraske could not use this method to determine the number of ferric ions complexing with glucose because of the high absorbances of ferric sulfate solutions compared to that of the complex (11). But the perchlorate anion was chosen for the present study because it has much less tendency than sulfate to complex with metal ions. So use of the limiting logarithms method to determine the number of ferric ions complexing with glucose and the other substrates was attempted.

Figure 69 gives the results obtained with glucose. The value obtained for the ferric ion coefficient (0.87) cannot be correct since m cannot be less

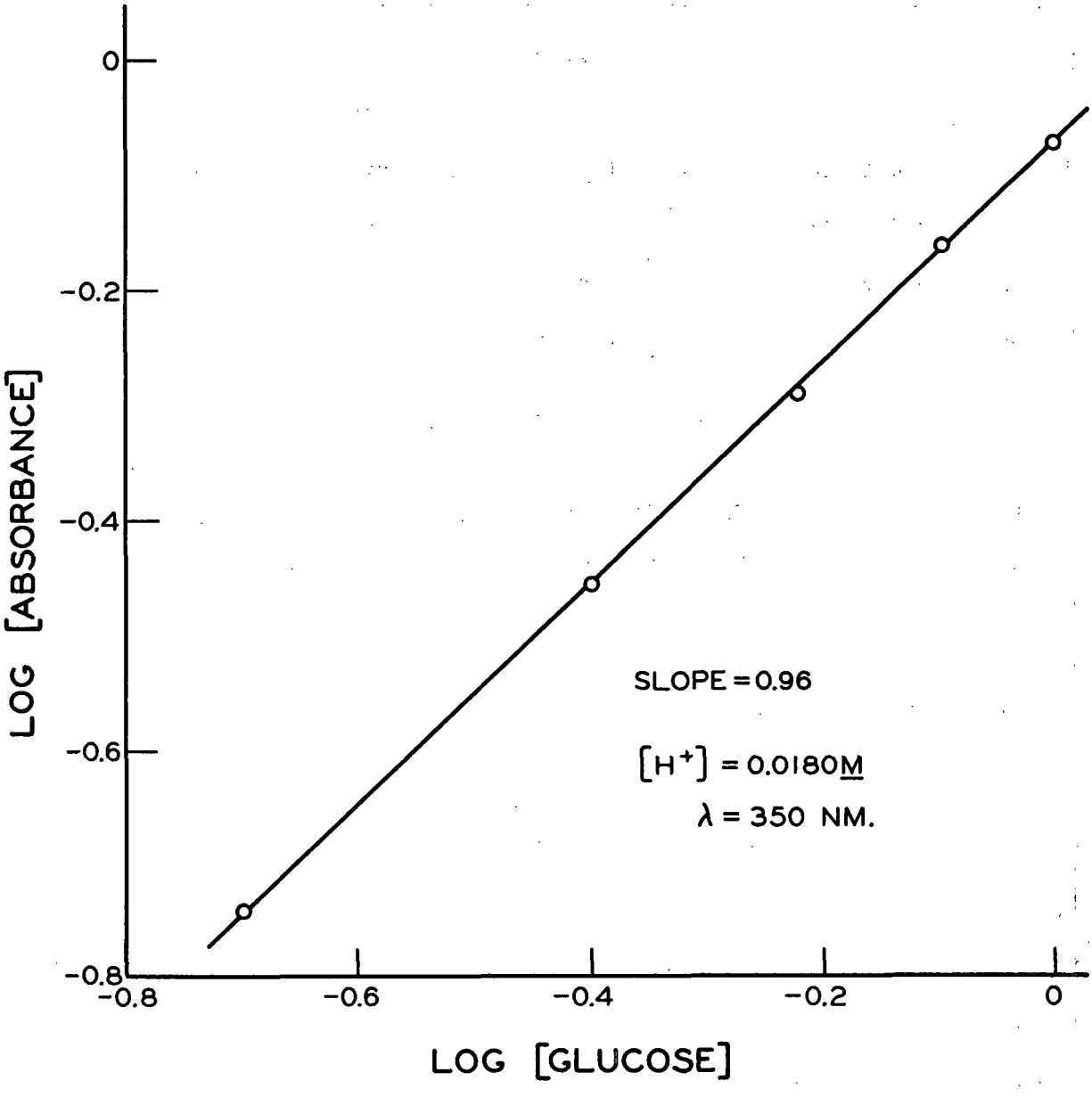

Figure 67. Limiting Logarithms Plot for the Glucose-Ferric Ion Complex (Varying Substrate Concentration)

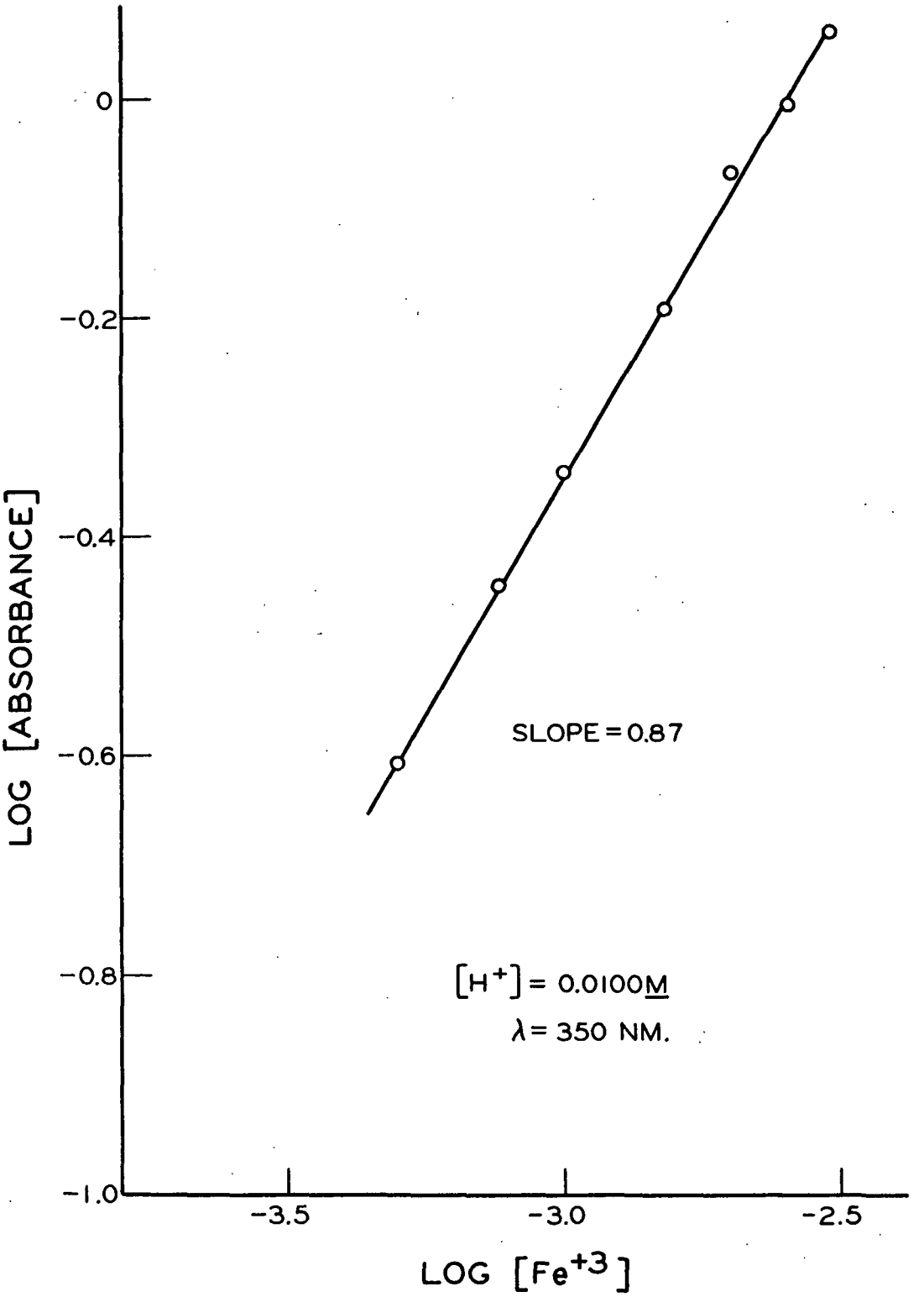

Figure 68. Limiting Logarithms Plots for Ferric Ion Complexes of trans-1,2-Cyclohexanediol, Glyceraldehyde, and Glycolaldehyde (Varying Substrate Concentration)

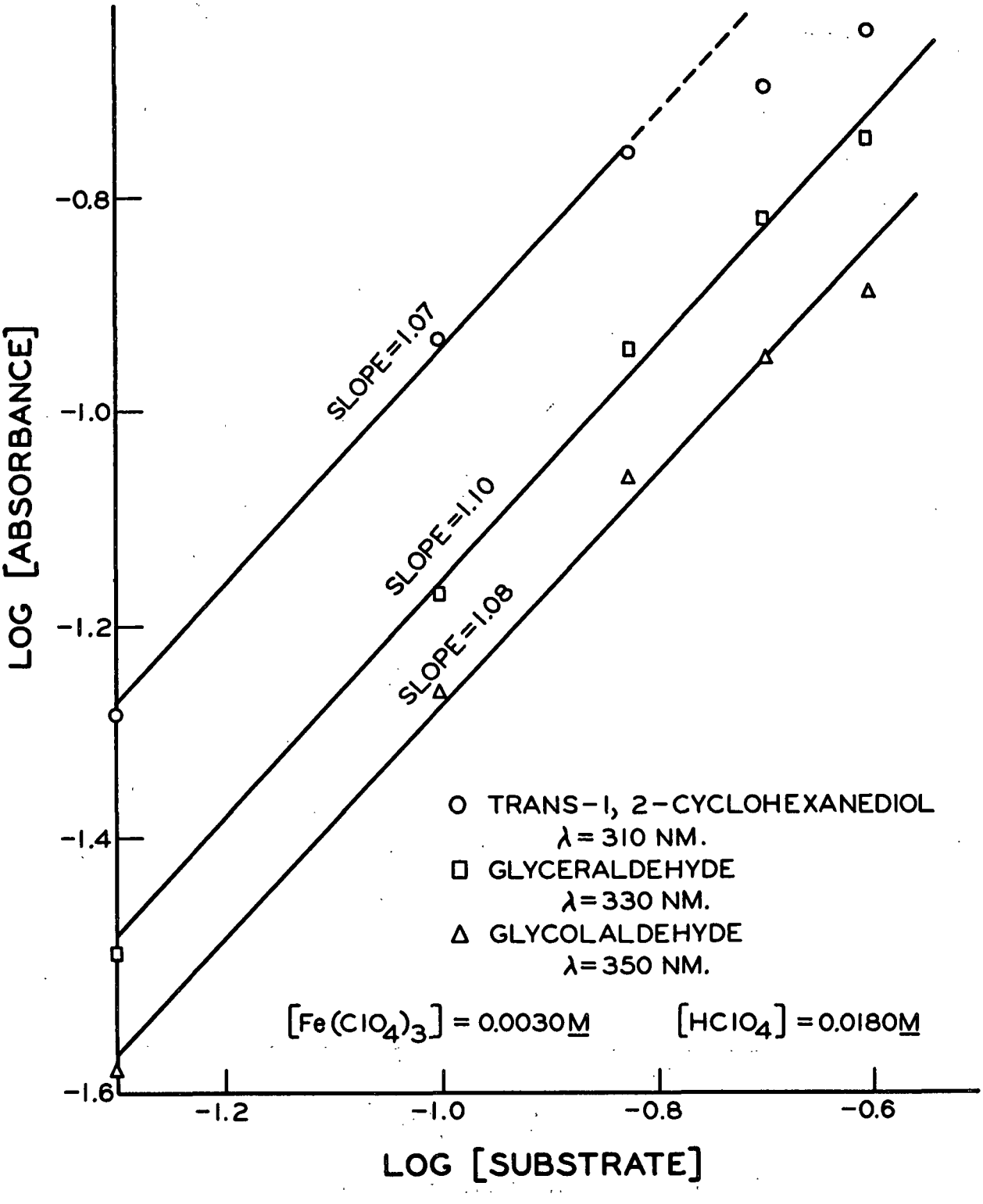

Figure 69. Limiting Logarithms Plot for the Glucose-Ferric Ion Complex (Varying Ferric Ion Concentration)

than one. Fractional values of 0.65, 0.67, and 0.72 were obtained for the ferric ion coefficients of trans-1,2-cyclohexanediol, glyceraldehyde, and glycolaldehyde complexes.

The explanation for these unreasonable results is probably that the method of limiting logarithms is not applicable under the conditions used. In the derivation of the limiting logarithms relationships, it was assumed that $[\underline{M}^+] \approx [\underline{M}_T^+]$. But in the experiments with glucose and the other substrates, concentrations were such that $[\underline{L}_T] >> [\underline{M}_T^+]$. Under these conditions, a significant proportion of $[\underline{M}_T^+]$ may be tied up in the complex so that $[\underline{M}^+] \not\approx [\underline{M}_T^+]$. If this is the case, use of the limiting logarithms relationship cannot be expected to give the correct value for $\underline{m}$, the stoichiometric coefficient of the metal ion.

On the other hand, a satisfactory estimate may be obtained for the ligand coefficient, $\underline{n}$, even under conditions where $[\underline{M}^+] \not\approx [\underline{M}_T^+]$. To see why this is so, consider the relationship,

$$\log A \propto m \log [M^+] + \log K + n \log [L]$$

which is valid for all concentrations of $\underline{M}^+$ and $\underline{L}$. When $[\underline{L}_T] >> [\underline{M}_T^+]$, $[\underline{L}] \approx [\underline{L}_T]$ because of the large excess of $\underline{L}$ compared with $\underline{M}^+$. Therefore,

$$\log A \propto m \log [M^+] + \log K + n \log [L_T].$$

If $[\underline{L}_T]$ is varied but $[\underline{M}_T^+]$ is held constant, $\log [\underline{M}^+]$ in the above relationship will vary in response to changes in $[\underline{L}_T]$. But, if $\underline{K}$ is not too large, variations in $\log [\underline{M}^+]$ will be small in comparison to variations in $\log [\underline{L}_T]$. In this case, the above relationship is approximated by the limiting logarithms relationship,

$$\log A \propto n \log [L_T] + C',$$

where $\underline{C}'$ is not truly constant, but varies slightly. This may explain why sensible answers were obtained for ligand coefficients by the limiting logarithms method.

It should be pointed out that the limiting logarithms method gives estimates close to unity for ligand coefficients in ferric ion complexes of trans-1,2-cyclohexanediol, glyceraldehyde, and glycolaldehyde, while Ardon's method suggests a coefficient of two. The reason for this discrepancy is uncertain, particularly since both methods indicate coefficients of unity for the glucose-ferric ion complex. As indicated earlier, the limiting logarithms approximation for the case where the ligand is present in great excess holds true only when the equilibrium constant, K, is relatively small. Perhaps this condition is not met in ferric ion complexes of trans-1,2-cyclohexanediol, glyceraldehyde, and glycolaldehyde, in which case the limiting logarithms method would give spurious results. For this reason, the coefficients obtained by the use of Ardon's method are considered to be more reliable than those from the limiting logarithms method.